

The Journal of Parasitology

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PROGRAM AND ABSTRACTS OF THE TWENTY-EIGHTH ANNUAL
MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS,
WISCONSIN UNIVERSITY, MADISON, WISCONSIN

September 7, 8, 9, 1953

PROGRAM¹

MONDAY MORNING SESSION, SEPTEMBER 7, 9:00 AM, 251 CHEMISTRY HALL.
E. R. BECKER, IOWA STATE COLLEGE, PRESIDING.

Read

1. A high incidence of blood parasites in a population of sooty grouse. (10 min.) (Lantern). J. R. ADAMS AND J. F. BENDELL, University of British Columbia.
2. Experimental leishmaniasis in the chinchilla. (10 min.) (Lantern). L. A. STAUBER, Rutgers University.
3. Observations on trichomonads of the digestive tract and nose of pigs. (10 min.) (2×2 lantern). D. M. HAMMOND AND P. F. FITZGERALD, Utah State Agricultural College and U. S. Bureau of Animal Industry.
4. Studies on the likelihood of transmission of bovine trichomoniasis by artificial insemination. (10 min.) (2×2 lantern, opaque projector). P. F. FITZGERALD, D. M. HAMMOND AND M. L. MINER, Utah State Agricultural College and U. S. Bureau of Animal Industry.
5. Observations on the passage of virulent *Trichomonas gallinae* through 119 successive domestic pigeons. (10 min.) (Lantern). R. M. STABLER, Colorado College.
6. The effect of cortisone in murine toxoplasmosis. (10 min.) (Lantern). L. JACOBS AND M. L. MELTON, National Microbiological Institute.
7. Measurement of metabolic activity of *Endamoeba histolytica* in cultures containing bacteria. (15 min.) (Lantern). J. L. BRADIN, JR. AND E. KUN, Tulane University Medical School.
8. The infection of *Anopheles quadrimaculatus*, a human malaria vector, with *Plasmodium cathemerium*, an avian malarial parasite, (15 min.) (Lantern). D. W. MICKS AND V. MCCOLLUM, University of Texas Medical Branch, Galveston.
9. Partial development *in vitro* of oocysts of *Plasmodium relictum*. (10 min.) (Lantern). G. H. BALL, University of California at Los Angeles.
10. The infection of duck and goose embryos by *Plasmodium berghei*, a mammalian malarial parasite. (15 min.) (Lantern). R. B. MCGHEE, The Rockefeller Institute for Medical Research. (Also by demonstration—see No. 72).

¹ An alphabetical author-index will be found at the end of the program. Extra copies of this supplement, and portraits of parasitologists, will be on sale at the meeting.

11. Experimental chemotherapy of *Plasmodium hexamerium* infection in ducks. (12 min.) (Lantern). R. D. MANWELL AND P. KHABIR, Syracuse University.

12. Glycogen studies on white rats infected with *Plasmodium berghei*. (15 min.) (Lantern). T. I. MERCADO AND T. VON BRAND, National Microbiological Institute.

13. Chemotherapy of experimental dourine (*Trypanosoma equiperdum* infection) (12 min.) (Lantern). A. PACKCHANIAN AND F. PINDAK, University of Texas Medical Branch, Galveston.

14. The epidemiology of intestinal parasites in Japanese family groups. (15 min.) (Lantern). D. E. WYKOFF, 406th. Medical General Laboratory, M. YOKOGAWA, Institute of Public Health, Tokyo, Y. KOMIYA, National Institute of Health, Tokyo, S. SUGIURA, Yamanashi Medical Research Institute, and L. S. RITCHIE, 406th. Medical General Laboratory.

By Title

15. The susceptibility of various inbred strains of mice to toxoplasmosis. L. JACOBS AND M. L. MELTON, National Microbiological Institute.

MONDAY AFTERNOON SESSION, SEPTEMBER 7, 2:00 PM, 251 CHEMISTRY HALL.
J. C. SWARTZWELDER, LOUISIANA STATE UNIVERSITY SCHOOL OF MEDICINE,
PRESIDING.

Read

16. The life history of *Azygia longa* (Leidy, 1851) (Trematoda: Azygiidae). (12 min.) (Lantern). E. I. SILLMAN, University of Michigan.

17. Life history of a strigeid trematode, *Hysteromorpha triloba* (Rudolphi, 1819) Lutz, 1931. (15 min.) (2×2 lantern). E. J. HUGHINS, South Dakota State College. (Also by demonstration—see No. 71).

18. Studies on the life histories of *Maritrema obstipum* (Van Cleave and Mueller, 1932) and *Levinsemiella* sp. (Trematoda: Microphallidae). (15 min.) F. J. ETGES, New York University.

19. Studies on encystation of larval digenetic trematodes. (15 min.) (2×2 lantern). F. J. KRUIDENIER, University of Illinois.

20. Experimental infections of Rhesus monkeys with *Schistosomatium douthitti* (Cort, 1914). (15 min.) (2×2 lantern). I. G. KAGAN, University of Pennsylvania.

21. Bisexual reproduction in the mother sporocyst of *Paragonimus kellicotti* (Trematoda). (15 min.) (Lantern). A. E. WOODHEAD, University of Michigan.

22. Observations made by the World Health Organization Schistosomiasis Team in the Philippines. (15 min.) (2×2 lantern). D. B. McMULLEN, Army Medical Service Graduate School, B. HUBENDICK, Naturhistoriska Riksmuseet (Stockholm), T. P. PESIGAN, Department of Health, Manila, and P. BIERSTEIN, W.H.O. Western Pacific Regional Office.

23. Nocturnal emergence of *Schistosoma mansoni* cercariae from *Australorbis glabratus* by inversion of light cycle and practical application. (15 min.) (Lantern). G. W. LUTTERMOSER, National Microbiological Institute.

24. Pulmonary manifestations of single sex *Schistosoma mansoni* infections in mice. (12 min.). W. B. DEWITT, National Microbiological Institute.

25. Penetration of definitive host skin by cercariae of *Schistosoma mansoni*. (15 min.) (2×2 lantern). M. A. STIREWALT, Naval Medical Research Institute.
26. Host-parasite relationships of *Schistosoma mansoni*. (15 min.) (Lantern). J. H. THOMPSON, JR., Section of Clinical Pathology, Mayo Clinic, Rochester, Minnesota.
27. Observations on immunity to superinfection with *Schistosoma mansoni* and *S. haematobium* in monkeys. (15 min.) (Lantern). H. E. MELENEY AND D. V. MOORE, New York University College of Medicine.
28. Studies on the nervous system of *Postharmostomum helici* (Leidy, 1847) Robinson, 1949 (Trematoda: Brachylaematidae). (5 min.) (Standard and 2×2 lantern). M. J. ULMER, Iowa State College.

By Title

29. Studies on schistosomiasis. IX. Studies on some ointments for protection against schistosomiasis mansoni. M. G. RADKE, G. W. HUNTER III, A. P. MOON, D. E. POTTS AND J. S. WILLIAMS, Fourth Army Medical Laboratory, B.A.M.C., Fort Sam Houston.
30. The influence of pH, temperature and light on the emergence of the cercariae of *Schistosoma japonicum* from *Oncomelania nosophora*. A. GUMBLE, Y. OTORI, L. S. RITCHIE AND G. W. HUNTER III, 406th. Medical General Laboratory, Tokyo.
31. The biology of a marine dermatitis-producing schistosome cercaria from *Batillaria minima* (Gmelin). L. R. PENNER, University of Connecticut.
32. The red-breasted merganser as a natural avian host of the causative agent of clam diggers' itch. L. R. PENNER, University of Connecticut.
33. Experimental infections of avian hosts with *Cercaria littoralinae* Penner, 1950. L. R. PENNER, University of Connecticut and the Biological Research Institute, San Diego.
34. The life history of *Entosiphonus thompsoni* Sinitsin, 1931 (Trematoda: Brachylaematidae). J. B. VILLELLA, University of Michigan.
35. The life history of *Ectosiphonus rhomboideus* Sinitsin, 1931 (Trematoda: Brachylaematidae) from the short-tailed shrew, *Blarina brevicauda*. J. B. VILLELLA, University of Michigan.
36. Life cycle of *Paramacroderoides echinus* Venard, 1941, a parasite of the Florida gar, *Lepisosteus platyrhincus*. R. B. HOLLIMAN AND W. H. LEIGH, University of Miami.
37. Notes on the life history of *Opisthorchis tonkai* Wallace and Penner, 1939 (Trematoda: Opisthorchiidae). E. I. SILLMAN, University of Michigan.
38. *Microtus pennsylvanicus pennsylvanicus*, a new final host for *Opisthorchis tonkai* Wallace and Penner, 1939 (Trematoda: Opisthorchiidae). E. I. SILLMAN, University of Michigan.
39. Studies on the life history of *Posthodiplostomum minimum* (Mac Callum, 1921). J. H. MILLER, New York University.
40. The entrance of the cercaria of *Echinoparyphium flexum* (Linton) Dietz, 1910, into tadpole kidneys. H. H. NAJARIAN, University of Michigan.
41. A partially-twinned daughter redia. H. H. NAJARIAN, University of Michigan.

42. An ancyliid snail, *Ferrissia novangliae*, a first intermediate host for trematodes. R. J. SMITH, University of Michigan.

43. *Fasciolopsis buski* (Lankester) in Central Thailand. E. H. SADUN, Division of International Health, U.S.P.H.S.

44. Preliminary report on the treatment of opisthorchiasis with Aralen (Chloroquine diphosphate) in Korat Province (Northeast Thailand). E. H. SADUN, Division of International Health, U.S.P.H.S., and C. CHAMNARNKITCH, Department of Health, Thai Ministry of Health.

45. A method of preparation of whole mounts of miracidia and cercariae. J. S. HUNT, University of Michigan.

MONDAY EVENING, SEPTEMBER 7, 6:30 PM, ROSE TAYLOR ROOM, KRONSAE HALL. DINNER AND COUNCIL MEETING, OFFICERS AND MEMBERS OF THE SOCIETY'S COUNCIL.

(All Past Presidents of the Society are cordially invited to attend both the dinner and the meeting)

TUESDAY MORNING SESSION, SEPTEMBER 8, 9:15 AM, 251 CHEMISTRY HALL. H. W. BROWN, COLUMBIA UNIVERSITY SCHOOL OF PUBLIC HEALTH, PRESIDING.

Read

46. *Bialovarium nocomis* n.g., n. sp. (Cestoda: Caryophyllaeidae) from the hornyhead chub, *Nocomis biguttatus* (Kirtland). (5 min.) (Lantern). J. H. FISCHTHAL, Harpur College, State University of New York.

47. Morphological observations on normal and triradiated *Taenia pisiformis* from the dog. (12 min.) (Lantern). E. E. BYRD AND F. W. FITE, University of Georgia.

48. The effects of *Taenia taeniaeformis* and *Amphimerus pseudofelineus* on the liver as shown by histochemical techniques. (10 min.) (2×2 lantern). R. M. LEWERT AND C. L. LEE, University of Chicago.

49. The size attained by *Hymenolepis diminuta* in different host species. (10 min.) (Lantern). C. P. READ AND M. VOGEL, University of California at Los Angeles.

50. Evaluation of field procedures related to molluscacidal applications. (10 min.) (Standard and 2×2 lanterns). J. R. C. FONSECA, J. E. WILLIAMS, R. E. FREYTAG AND L. S. RITCHIE, 406th Medical General Laboratory, Tokyo.

51. Information pertinent to destruction of eggs and newly hatched of *Oncomelania nosophora* by molluscacides. (10 min.) (Standard and 2×2 lanterns). L. S. RITCHIE, Y. OTORI, J. R. C. FONSECA AND J. E. WILLIAMS, 406th Medical General Laboratory, Tokyo.

52. Determination of the relative efficiencies of molluscacides by means of field-plot dilution tests. (10 min.) (Standard and 2×2 lanterns). J. E. WILLIAMS, R. E. FREYTAG, J. R. C. FONSECA AND L. S. RITCHIE, 406th General Medical Laboratory, Tokyo.

53. Studies on the Lone Star tick. II. Preliminary plot tests of potential acaricides (Tickicides). (12 min.) (Lantern). A. A. THERRIEN, G. W. HUNTER III, A. P. MOON, A. L. ADAMS, D. E. POTTS, M. G. RADKE, J. S. WILLIAMS AND J. E. WEBB, JR., Fourth Army Medical Laboratory, B.A.M.C., Fort Sam Houston.

By Title

54. The synthesis of amino acids from ammonia in *Hymenolepis diminuta*. J. DAUGHERTY, The Rice Institute.
55. The metabolism of sulfur amino acids in *Hymenolepis diminuta*. S. GARSON AND J. DAUGHERTY, The Rice Institute.
56. Vitamin B₁₂ and desoxyribonucleic acid relationship in *H. diminuta*. W. G. DACRES, W. NYBERG AND J. DAUGHERTY, The Rice Institute.
57. Vitamin B₁₂ uptake by *Hymenolepis diminuta*. W. NYBERG, The Rice Institute and the University of Helsingfors. (Introduced by J. DAUGHERTY.)
58. Auto-reinfection in white mice resulting from infection by *Hymenolepis nana*. D. HEYNEMAN, The Rice Institute.
59. Cross-immune protection against *Hymenolepis diminuta* by *H. nana* in white mice. D. HEYNEMAN, The Rice Institute.
60. Tetraphyllidean cestodes from the Eastern Pacific. C. G. ALEXANDER, University of California at Los Angeles.
61. The biology of *Oochoristica* and the status of linstowiine cestodes. R. E. MILLEMAN AND C. P. READ, University of California at Los Angeles.
62. *Cysticercus fasciolaris* in the Syrian Hamster. W. W. WANTLAND, Illinois Wesleyan University.
63. *Cysticercus fasciolaris* in the wild rat and the development of this strobilocercus in the white rat. W. W. WANTLAND, Illinois Wesleyan University.
64. The occurrence of a nymphal linguatulid (Arthropoda: Pentastomida) in the lungs of the domestic cat. L. M. YUTUC, University of the Philippines.
65. Statistical analysis applied to numbers of worms recovered by post mortem examinations. J. R. EGERTON, Kansas State College.

Presidential Address, 230 SERVICE MEMORIAL INSTITUTE

66. How Parasites Tolerate Their Hosts. (50 min.) ELERY R. BECKER, Iowa State College.
(In the absence of the President, C. B. Philip, who is in the Near East, the President-elect is serving as presiding officer at this meeting. Dr. Philip will give the address in 1954.)

TUESDAY AFTERNOON SESSION, SEPTEMBER 8, 2:00 TO 4:00 PM, 303 SERVICE MEMORIAL INSTITUTE.

By Demonstration

67. Observations on the lethal action of polyborate on swine kidney-worm (*Stephanurus dentatus*) larvae in soil. J. E. ALICATA, University of Hawaii. (also read—see No. 89)
68. Coccidiosis of mammary tissue in the water shrew, *Sorex palustris navigator*, R. M. CABLE, Purdue University, AND C. H. CONAWAY, University of Wisconsin.
69. Contributions to the life cycles of gorgoderid trematodes. W. H. COIL, Ohio State University.
70. *Cercaria tiogae*, a new rhopalocercous form from the clam, *Alasmidonta varicosa* (Lamarck). J. H. FISCHTHAL, Harpur College, State University of New York.

71. Life history of a strigeid trematode, *Hysteromorpha triloba* (Rudolphi, 1819) Lutz, 1931. E. J. HUGHINS, South Dakota State College. (also read—see No. 17)

72. The infection of duck and goose embryos by *Plasmodium berghei*, a mammalian malarial parasite. R. B. MCGHEE, The Rockefeller Institute for Medical Research. (also read—see No. 10)

73. New host and distribution records for the trematode genus *Braunina* Heider, 1900. A. MCINTOSH, U. S. Bureau of Animal Industry.

74. Exogenous proliferation in a larval taeniid (Cestoda: Cyclophyllidea) from the body cavity of Peruvian rodents. M. VOGEL, University of California School of Medicine, Los Angeles.

TUESDAY EVENING, SEPTEMBER 8, 6:30 PM. PICNIC AT DEVIL'S LAKE.

Buses will leave the campus at 4:15 and the picnic is at 6:30. Devil's Lake is about 40 miles from Madison. (Tickets must be arranged for at the time of registration).

WEDNESDAY MORNING SESSION, SEPTEMBER 9, AM, 251 CHEMISTRY HALL.

G. W. WHARTON, DUKE UNIVERSITY, PRESIDING.

Read

75. A method for revealing cuticular structures of nematodes. (10 min.) (2×2 lantern). J. FELDMESSER AND A. L. TAYLOR, Division of Nematology, Bureau of Plant Industry, Hicksville, N. Y.

76. Infectivity and host-response in root-knot nematode infections. (15 min.) (Lantern). V. H. DROPKIN, Naval Medical Research Institute.

77. Some observations on the monoxenic cultivation of certain rhabditid nematodes. (5 min.) E. C. DOUGHERTY, University of California at Berkeley.

78. Some observations on the axenic cultivation and attempted cultivation of certain rhabditid nematodes. (5 min.) E. C. DOUGHERTY, University of California at Berkeley.

79. Axenizing and monoxenizing soil nematodes. (5 min.) E. C. DOUGHERTY, University of California at Berkeley.

80. Infectivity for Japanese beetle grubs retained by *Neoplectana glaseri* after seven years axenic culture. (15 min.) (Lantern). N. R. STOLL, The Rockefeller Institute for Medical Research.

81. Prenatal infection of a calf with the nematode, *Neoascaris vitulorum*. (10 min.). H. HERLICH AND D. A. PORTER, U. S. Regional Animal Research Laboratory, Auburn, Ala.

82. Transmission of trichinae to swine through feces. (15 min.). L. A. SPINDLER, Bureau of Animal Industry.

83. Occurrence of *Amidostomum* in Canada geese. (10 min.). C. M. HERMAN, Patuxent Research Refuge, and E. E. WEHR, Bureau of Animal Industry.

84. The incidence of some common canine intestinal parasites. (10 min.). (Lantern). F. A. EHRENFORD, Pitman-Moore Co., Indianapolis.

85. An experiment on the pathogenic interaction of *Haemonchus contortus* and *Nematodirus spathiger* in lambs. (15 min.) (Lantern). K. C. KATES AND J. H. TURNER, Bureau of Animal Industry.

86. The effect of cortisone on the development of the immune response in the white rat to *Nippostrongylus muris*. (10 min.). P. P. WEINSTEIN, National Microbiological Institute.

87. The effect of pteroylglutamic acid, vitamin B₁₂, and related compounds on *Ascaridia galli* infections in chicks. (15 min.) (Lantern). G. BRODY, Michigan State College.

WEDNESDAY NOON, SEPTEMBER 9, 12:30 PM, CONGREGATIONAL CHURCH, 1609 UNIVERSITY AVE. ANNUAL LUNCHEON AND BUSINESS MEETING OF THE SOCIETY. E. R. BECKER, PRESIDING.

(get tickets at time of registration)

WEDNESDAY AFTERNOON SESSION, SEPTEMBER 9, 251 CHEMISTRY HALL. P. C. BEAVER, TULANE UNIVERSITY, PRESIDING.

Read

88. Certain net effects of the free-choice administration of phenothiazine to sheep. (15 min.) (Lantern). A. O. FOSTER, Bureau of Animal Industry.

89. Observations on the lethal action of polyborate on swine kidney-worm (*Stephanurus dentatus*) larvae in soil. (15 min.) (Lantern). J. E. ALICATA, University of Hawaii. (also by demonstration—see No. 67)

90. The effects of some phenothiazine derivatives and analogs on horse strongyle developmental stages in feces. (15 min.) (Lantern). N. D. LEVINE AND V. IVENS, University of Illinois.

91. Illustration of critical phases in the development of *Litomosoides carinii*, filarial parasite of the cotton rat. (10 min.) (Motion picture). E. M. MACDONALD AND J. A. SCOTT, University of Texas Medical Branch, Galveston.

92. Recent developments in methods for maintaining and transmitting *Litomosoides carinii* in the laboratory. (15 min.) (Lantern). J. A. SCOTT AND E. M. MACDONALD, University of Texas Medical Branch, Galveston.

93. Infections resulting from three *Necator americanus* larvae. (15 min.) (2×2 lantern). P. C. BEAVER, Tulane University.

94. Further observations on the incidence and the inconstancy of laboratory findings in enterobiasis. (12 min.) (Lantern). W. H. HEADLEE, Indiana University School of Medicine.

95. Chromatography as an aid to the taxonomy and phylogeny of animal parasites. (15 min.) (2×2 lantern). G. W. RAWSON, Ciba Pharmaceutical Products, Inc., Summit, N. J.

96. The influence of antibiotics in feed on sarcoptic mange in pigs. (15 min.) (Lantern). D. A. SHORB, Bureau of Animal Industry.

By Title

97. Intermediate hosts in *Ascaris* infections. J. F. A. SPRENT, Veterinary School, Yeerongpilly, Brisbane, Australia.

98. Clinical parasitism in cattle in Georgia. J. S. ANDREWS, D. J. JONES, Bureau of Animal Industry, and W. L. SIPPLE, Georgia Coastal Plain Experiment Station.

99. Preliminary report on endoparasites of beef cattle in Kansas. L. W. DEWHIRST, M. F. HANSEN AND J. E. ACKERT, Kansas State College.
100. Further studies of the value of phenothiazine, free-choice, against infections of *Nematodirus* and *Haemonchus* in lambs. J. H. TURNER AND M. L. COLGLAZIER, Bureau of Animal Industry.
101. Photosensitization keratitis in young goats following treatment with phenothiazine. F. D. ENZIE AND G. E. WHITMORE, Bureau of Animal Industry. (Introduced by A. O. FOSTER)
102. Toluene (methylbenzene) against intestinal nematodes in dogs and cats. F. D. ENZIE AND M. L. COLGLAZIER, Bureau of Animal Industry.
103. Effects of certain anthelmintics on lumen and tissue phase larvae of *Ascaridia galli* (Schränk). M. F. HANSEN, B. R. B. PERSAUD AND J. E. ACKERT, Kansas State College.
104. A histopathologic study of the anterior small intestine of immunized and non-immunized mice infected with *Trichinella spiralis*. J. E. LARSH, JR., University of North Carolina, and G. J. RACE, Duke University School of Medicine.
105. Passive transfer of resistance to *Trichinella spiralis* in mice. J. R. HENDRICKS, University of North Carolina.
106. The effect of water starvation on the natural resistance of mice to *Trichinella spiralis*. C. H. CAMPBELL, University of North Carolina.
107. The use of the collodion particle agglutination test for detecting antibodies formed in response to *Trichinella spiralis* infection. C. H. CAMPBELL, University of North Carolina.
108. A comparison in mice of the infectivity of *Trichinella spiralis* larvae in normal saline solution and in a solution of nutrient broth and gelatin. J. E. LARSH, JR., J. W. MCKENZIE, B. G. GREENBERG AND C. H. CAMPBELL, University of North Carolina.
109. The effect of storage at low temperatures on the infectivity of *Aspiculuris tetraptera* eggs. K. F. CHAN, Columbia University School of Public Health.
110. *In vitro* culture of the larvae of *Ascaris lumbricoides suum*. T. D. PITTS AND G. H. BALL, University of California at Los Angeles.
111. Preliminary survey of helminthic diseases in Thailand. E. H. SADUN, Division of International Health, U.S.P.H.S.

WEDNESDAY EVENING, SEPTEMBER 9, 9:00 TO 12:00 PM,
BIOLOGISTS' SMOKER—Admission by badge of registration.

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ABSTRACTS

1. *A High Incidence of Blood Parasites in a Population of Sooty Grouse.* JAMES R. ADAMS AND J. F. BENDELL, The University of British Columbia.

Fowle (Science, 103: 708-709, 1946) reported the occurrence of *Haemoproteus* sp., *Leucocytozoon* sp., *Trypanosoma* sp. and *Microfilaria* sp. in *Dendrogapus obscurus fuliginosus* (Ridgway) from two localities on Vancouver Island, British Columbia, and gave figures on the incidence and degree of infection in 44 birds examined in 1943-44. Our observations on over 300 birds at Campbell River during the summers of 1950-53 indicate a very much higher incidence of parasitism with these species.

Of 252 adult birds, *Haemoproteus* occurred in 92%, *Leucocytozoon* in 87%, *Trypanosoma* in 76% and *Microfilaria* in 82%. Quadruple infections with these species were found in 55% of 92 adult birds in 1950 and in 61% of 82 adults in 1951. During 1952, smears made in April and May revealed fewer infections than those made in June, July and August. One hundred percent of 59 adult birds captured in the latter period carried *Haemoproteus*.

Chicks examined in 1950 and 1951 were less heavily infected (*Haemoproteus* 80%, *Leucocytozoon* 46%, *Trypanosoma* 17%, *Microfilaria* 0).

One *Haemoproteus* infection gave a count of more than 500 parasites per 1000 r.b.c.; several approximated 200 per 1000 r.b.c. *Leucocytozoon* infections showed 1 or 2 parasites per 1000 r.b.c., while *Trypanosoma* and *Microfilaria* infections were less than one per 1000 r.b.c.

2. *Experimental Leishmaniasis in the Chinchilla.* L. A. STAUBER, Rutgers University, The State University of New Jersey.

The chinchilla (*Chinchilla lanigera*) has been found susceptible to the Khartoum strain of *Leishmania donovani*. Even on the first passage from the infected hamster, by intraperitoneal inoculation of spleen suspension, a heavy infection was obtained. Subsequently passed by either intravenous or intraperitoneal routes, fatal infections have been produced, death occurring in from one and one-half to three months after inoculation. Now in its fifth passage in the chinchilla, the organisms are still pathogenic for the hamster as well. The infection in the chinchilla does not appear to differ significantly from that in other susceptible animals. Hepatomegaly and marked splenomegaly are characteristic of the infection in the chinchilla. Parasite densities in impression smears of the spleens of animals in the terminal stages of the infection have reached as high as nine leishmaniform parasites per spleen cell nucleus.

3. *Observations on Trichomonads of the Digestive Tract and Nose of Pigs.* DATUS M. HAMMOND AND PAUL R. FITZGERALD, Agricultural Experiment Station, Utah State Agricultural College in collaboration with the Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture.

Using a modified Plastring medium, cultures of trichomonads from the cecum, stomach and nose of pigs have been established. Cultures of nasal trichomonads have also been obtained from Dr. L. A. Spindler, Zoological Division, B.A.I., Beltsville, Md. Living trichomonads from all of these cultures, and directly from the cecum of pigs, have been studied with the aid of the phase contrast microscope. Stained preparations made with the protargol and iron-haematoxylin methods were also studied.

All these trichomonads typically had 3 anterior flagella. Those directly from the cecum had relatively short, high undulating membranes 1/2 to 2/3 length of the spheroidal to ovoidal body and a relatively rapid rate of movement. In culture, cecal trichomonads underwent a modification involving elongation of the body, and some degree of change in the other features mentioned. In the cultures of nasal trichomonads, the body shape was typically elongate, the undulating membrane relatively low and nearly as long as the body, and the rate of movement was relatively slow. The appearance and behavior of trichomonads in cultures from the stomach resembled trichomonads from the cecum more closely than those from the nose but there were differences in characteristics of growth of the cultures.

The trichomonad from the cecum is evidently *Trichomonas suis*, but further work is required to determine whether the trichomonads from the stomach and nose belong to the same or to different species.

4. *Studies on the Likelihood of Transmission of Bovine Trichomoniasis by Artificial Insemination.* PAUL R. FITZGERALD, DATUS M. HAMMOND, AND MERTHYR L. MINER, Agricultural Experiment Station, Utah State Agricultural College in collaboration with the Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture.

Three series of trials were conducted to determine the duration of survival of *Trichomonas*

foetus under conditions currently used for dilution and storage of semen. In each trial there were 5 vials, each with 0.5 ml. fluid containing *T. foetus* and 10 ml. of egg-yolk sodium citrate mixture with or without antibiotics, or of saline solution or culture medium in the controls. The vials were kept at 39° F. and examined daily until trichomonads had disappeared.

In a series of 3 trials the average duration of survival of trichomonads from cultures in buffer mixture with 6 mg. sulfanilamide, 1000 units penicillin, 1.0 mg. streptomycin and .05 ml. semen per ml. was 8.7 days as determined by culture method; average duration of sperm activity was 7.3 days. In another series of 7 trials, similar except that no semen was added, the average duration of survival of trichomonads from vaginal samples from an infected heifer was 9.8 days as determined by direct examination. In a series of 14 trials, in half of which concentration of antibiotics was halved, the average duration of survival of trichomonads from cultures was 5.9 days.

In a study of the results of insemination of 32 females with diluted semen from an infected bull no cases of infection were found and the rate of conception was similar to that for uninfected bulls.

It is concluded that transmission of bovine trichomoniasis is possible by methods of artificial insemination currently in use, but that the rate of transmission is probably very low.

5. *Observations on the Passage of Virulent Trichomonas gallinae through 119 Successive Domestic Pigeons.* R. M. STABLER, Colorado College.

The extremely virulent Jones' Barn strain of *Trichomonas gallinae* was placed successively in 119 clean domestic pigeons from 14.VII.50 to 19.V.53. The birds were: 1 roller, 4 kings, 113 homers, and 1 homer-king cross. The age groups were: 24 adults (6 mos. and over), 38 juveniles (8 wks. to 6 mos.), and 52 squabs (5 to 8 wks.). The site in the donor from which the trichomonads were secured for placing in the recipients' mouths were: mouth (17), liver (88), mouth and liver (7), mouth and brain (1), and lung (1). Ninety donors were used shortly after their death; 19 were dead approximately 24 hrs. before their flagellates were removed for infection. Of the 119 birds, 114 died of trichomoniasis; 5 survived. Death occurred at 8.2 da. (av.), with a range of 4-18 da. The five survivors, which were in continuous isolation, were: 4 homers, 1 king. Three of these were still positive at autopsy, at 146, 283, and 620 da. Two were negative at autopsy, at 282 and 482 da. One of the latter was last known to be positive at 18, the other at 391 da. Trichomonads from the bird which was still positive when posted at 620 da. were given to 5 clean homers at 602 da. All five died (3 at 7 da., 1 at 9 da., and 1 at 11 da.).

6. *The Effect of Cortisone in Murine Toxoplasmosis.* LEON JACOBS AND MARJORIE L. MELTON, National Institutes of Health, Bethesda, Maryland.

Mice weighing about 18 grams, which survived for 7 weeks following inoculation with an avirulent strain (113) of *Toxoplasma* suffered no exacerbation of the infection when they were administered cortisone subcutaneously in doses of 0.125 mg. or 0.25 mg. daily for as long as 17 days. In another experiment cortisone was administered daily, starting day - 3, in doses of 0.25 mg. or 1.0 mg. subcutaneously, to 15-gram mice infected with 100 toxoplasmas of the avirulent strain. In this experiment, these doses of cortisone were lethal to a high percentage of the control mice and therefore it is difficult to attribute the greater number of deaths which occurred in the treated group, vs. those that received saline, to a more intense *Toxoplasma* infection. However, it was noted that treated mice which died late after infection showed many parasites in the liver and spleen on microscopic examination. This is in contrast to the ordinary course of infection with this *Toxoplasma* strain; mice dying late after infection usually die of encephalitis, with many parasites visible in the brain and only rarely seen in the liver and spleen. In further experiments, mice were infected with large numbers of toxoplasmas of the same strain and given 2.0 mg. cortisone on days - 1, 0, and 3. While the results were not always consistent, in general more cortisone-treated mice died than did controls, and their survival time was shortened.

7. *Measurement of Metabolic Activity of Endamoeba histolytica in Cultures Containing Bacteria.* JOHN L. BRADIN, JR., AND ERNEST KUN, Tulane University, New Orleans, La.

The products of metabolism of *E. histolytica* in cultures containing bacteria can be measured if the quantitative contributions made by each of the two types of organisms are determined. The production of carbon dioxide and hydrogen sulfide from various sugars and sulfur substrates has been measured under anaerobic conditions. It has been shown that gas production both by the bacteria and by the amebas is correlated with the numbers of organisms used. Consequently a mixture can be adjusted to contain large numbers of amebas and relatively small numbers of bacteria, thus minimizing the masking effect of bacterial activity. Killing the

amebas by quick freezing and thawing in an acetone-dry ice mixture is reflected in a loss of metabolic activity ascribed to the amebas, while leaving the concomitant bacterial activity unimpaired as compared with unfrozen bacterial controls. Using 400,000 amebas of Clone 22 with a mixed undetermined bacterial flora from 24-hour-old cultures in aqueous egg yolk infusion medium (Balamuth, 1946), 340 ml. of carbon dioxide have been measured in experiments lasting 90 minutes in the Warburg apparatus.

Gas production has been studied using a variety of sugars and sulfur-containing compounds as substrates. Results indicate that of nine sugars studied, glucose, mannose and galactose give the largest amounts of carbon dioxide.

Of twelve sulfur substrates, cysteine has given the best results in terms of hydrogen sulfide production.

Using preparations of starved amebas, to exhaust endogenous substrates, an interdependence between CO_2 and H_2S production has been demonstrated.

8. *The Infection of Anopheles quadrimaculatus, a Human Malaria Vector, with Plasmodium cathemerium, an Avian Malaria Parasite.* DON W. MICKS AND VENICE MCCOLLUM, University of Texas Medical Branch, Galveston, Texas.

In a series of ten experiments which involved a total of 120 *Anopheles quadrimaculatus* 31 or 25.8 percent of these mosquitoes were infected with *Plasmodium cathemerium* for the canary. The number of oocysts per stomach varied from 3 to 600, averaging 33. Even though the oocysts matured and appeared normal in every respect, sporozoites have not yet been found in the salivary glands of *A. quadrimaculatus* kept as long as 21 days after an infectious blood meal. Several batches of *Culex quinquefasciatus* fed simultaneously with the *Anopheles* revealed infection rates of 75 to 93 percent.

9. *Partial Development in vitro of Oocysts of Plasmodium relictum.* GORDON H. BALL, University of California at Los Angeles.

Attempted cultivation *in vitro* of oocysts of *Plasmodium relictum* attached to the stomach wall of *Culex tarsalis* (Ball, 1947, 1948) resulted in persistence of contraction of the gut of adult mosquitoes for a maximum of seven days and the maintenance of oocysts in an apparently normal condition for as long as 20 days, although little or no development of oocysts took place.

As a result of improved cultural conditions, it has proved possible to maintain contractility of adult mosquito digestive tract preparations for at least five weeks. Oocysts attached to contracting stomachs grew for four or five days, increasing in diameter up to three times their original size. The rate of growth, however, was less than that of *in vivo* controls. This development occurred in cultural medium which had been improved by approximating the chemical composition of insect tissue and body fluids, by increasing the proportion of chick serum, and by carrying on the cultivation initially at lower temperature. Further work is proceeding to produce conditions which will result in continued development to maturity of the attached oocysts.

10. *The Infection of Duck and Goose Embryos by Plasmodium berghei, a Mammalian Malarial Parasite.* R. BARCLAY MCGHEE, The Rockefeller Institute for Medical Research.

Considerable numbers of parasites were observed in the erythrocytes of duck and goose embryos 24 hours after the intravenous injection of mouse blood containing *Plasmodium berghei*. Similar injections into chick embryos have thus far failed to elicit visible invasion of avian cells. In embryos of both the duck and goose the number of parasites was less on succeeding days until at 4 days only an occasional parasite was observed. Particularly outstanding was the variance in morphology of *P. berghei* from that seen in the mouse cell. In more mature avian erythrocytes the parasite was a slender band which hugged the periphery of the cell, while in basophilic erythroblasts frank rings were formed which lay well within the cytoplasm of the blood cell. Segmentation occurred within 42 hours with the formation of from 9 to 16 merozoites. Immediately following segmentation there was a precipitous drop in parasite numbers with the eventual termination of visible parasitemia.

11. *Experimental Chemotherapy of Plasmodium hexamerium Infections in Ducks.* REGINALD D. MANWELL, AND PARVIZ KHABIR, Syracuse University.

Plasmodium hexamerium, an avian malaria parasite fairly common in some of the smaller passerine birds, produces good infections in ducks. It belongs to a group of avian malaria parasites, relatively small in size and characterized by a moderate, but very persistent, parasitemia. Little experimental work has been done on any member of the group thus far, and the present study was attempted largely for this reason. Results with quinine, quinacrine, pamaquine and primaquine have already been reported. To these may now be added paludrine, chloroquine,

daraprim, pentaquine, and isopentaquine. The treatment period for each drug was a week, two doses 12 hours apart being given daily. Two trials of each drug were made, one during the patent period, and the other beginning 12 hours following initial infection. Infections were all blood-induced. The total number treated with the 5 last-named drugs was 112, with 56 controls. Birds treated during the patent and prepatent periods were equal in number.

Only pentaquine and isopentaquine proved capable of sterilizing this type of malaria in the duck, and this not in every case. (With the former drug, 1 case in 24 relapsed; with the latter, 2 of 16). Dosage was 0.00221 mg. per gm. of body weight for pentaquine; 0.00177 per gm. for isopentaquine. The other 3 drugs tested failed to sterilize any of the treated birds, and it was noted that with paludrine and daraprim gametocytes proved resistant, some of these stages being evident in the blood of most of the experimental birds even after treatment stopped. This was particularly true with paludrine. Dosage for each of these 3 drugs was: chloroquine, 0.0224 mg. per gm.; paludrine, 0.0315 mg. per gm.; daraprim, 0.002 mg. per gm.

12. *Glycogen Studies on White Rats Infected with Plasmodium berghei*. TERESA I. MERCADO AND THEODOR VON BRAND, National Institutes of Health, National Microbiological Institute, Bethesda, Maryland.

Determinations of the liver glycogen of female white rats infected with *Plasmodium berghei* showed a progressively decreasing glycogen content with increasing parasitemia. The carcass glycogen, on the contrary, stayed unchanged throughout the greater part of the infection; it decreased only in highly parasitized animals. Since infected animals consumed less food than healthy ones, control experiments were set up in which the uninfected rats were allowed to consume only as much food as the infected ones. These experiments showed that the entire loss of carcass glycogen and part of the loss of liver glycogen can be explained on the basis of semi-starvation. There was, however, a residual loss in liver glycogen that must be ascribed to a deficient liver function. This assumption is justified because further experiments showed that less glycogen was formed in the livers of infected than normal rats when they were given large amounts of sugar either orally or intravenously.

13. *Chemotherapy of Experimental Dourine (Trypanosoma equiperdum infection) in Mice (Mus musculus)*. A. PACKCHANIAN AND F. PINDAK, Department of Bacteriology and Laboratory of Microbiology, The School of Medicine, The University of Texas, Galveston, Texas.

200 mice, *Mus musculus*, experimentally infected with *Trypanosoma equiperdum* were treated with 21 new nitrofurantoin compounds. Out of 21 compounds, thirteen were without any therapeutic effect in spite of daily treatment with the maximum tolerance dose. The trypanosomes increased in the circulating blood of the animals rapidly and all animals were found dead within five days. The remaining eight compounds had a definite therapeutic effect on experimental trypanosomiasis in mice; the flagellates disappeared from the circulating blood of the experimental animals following one to three treatments. With a few of the drugs of this group the seemingly therapeutic effects were apparently only suppressive. The remaining four nitrofurantoin compounds produced an "absolute cure." The apparently cured mice were under observation for several weeks and none of these animals showed any relapse of trypanosomes as determined by frequent blood examinations microscopically and by animal inoculation tests.

Control, experimentally infected but untreated animals all died from trypanosomiasis within four to five days.

14. *The Epidemiology of Intestinal Parasites in Japanese Family Groups*. D. E. WYKOFF, 406th Medical General Laboratory, Tokyo; M. YOKOGAWA, Institute of Public Health, Tokyo; Y. KOMIYA, NIH, Tokyo; S. SIGIURA, Yamanashi Medical Research Institute; and L. S. RITCHIE, 406th Medical General Laboratory, Tokyo.

Members of 170 families distributed in four villages in Yamanashi Prefecture, Japan, were examined five times with the formalin-ether technic for intestinal parasites. In these villages, selected because of a high prevalency of protozoa, the incidence of *Endamoeba histolytica* varied from 13-29%. Two of the villages were treated for protozoa and, when subsequently examined after 7 months, it was noted that *E. histolytica* had increased from 0.9 to 2.2% in one village and from 1.9 to 4.1% in the other. The untreated villages are being examined over an 18 month period for evidence of spontaneous elimination of protozoa.

Correlation of age with the incidence of amebiasis showed a progressive, though not uniform, increase until age 60, after which there was a decline. Females were more frequently infected than males in a ratio of 1 to 1.53. Examinations of data were made to determine whether the food-handler is a primary factor in establishing the high incidence of infection. The occurrence of *E. histolytica* in families where the primary food-handler was infected has

been compared to families where a nonfood-handler was infected. A third category of comparison was introduced by selecting one person from each family by chance and determining the infection rate for families of those harboring *E. histolytica*. The data suggest that transmission of *E. histolytica* by food-handlers is not a primary factor in establishing the high rate of infection. Additional studies are being conducted on the various transmission tendencies of intestinal protozoa, including the role played by flies and the effect of contaminated water supplies.

15. *The Susceptibility of Various Inbred Strains of Mice to Toxoplasmosis*. LEON JACOBS AND MARJORIE L. MELTON, National Institutes of Health, Bethesda, Maryland.

Comparisons of the susceptibility of inbred mouse strains to the RH strain of *Toxoplasma gondii* revealed very little difference. The highly virulent RH strain regularly caused death of the mice; the only criterion was the survival time. Mouse strains A/LN, C57BL/6JN, C57BN/cdJN, BALB/cAnN, and their hybrids CAF₁ (BALB×A/LN) and LAF₁ (C57BN×A/LN) were tested. All except C57BL exhibited the same susceptibility as the general purpose NIH mice. C57BL mice showed only a slightly greater tolerance; their survival time, was, on the average, 1 day longer than that of general purpose mice. In limited tests with other highly pathogenic strains of *Toxoplasma* there was also no difference observed in the susceptibility of the BALB and A/LN mouse strains and their hybrid, compared among themselves and with general purpose mice. On the other hand, when a *Toxoplasma* strain less virulent than the RH strain was used (strain 113) the CAF₁ hybrid showed a much higher rate of survival than general purpose mice. However, the survivors evidenced no more immunity to RH challenge than did the surviving general purpose mice.

16. *The Life History of Azygia longa (Leidy, 1851) (Trematoda: Azygiidae)*. E. I. SILLMAN, University of Michigan.

Azygia longa has been recorded from the stomachs of various freshwater fish, e.g., esocids, centrarchids, percids, anguillidids, and salmonids in northeastern North America. It was reported once from a marine fish, *Trichiurus lepturus*, by Linton (1940, Proc. U. S. Nat. Mus., 88, No. 3078, 105-107) from the Woods Hole region, Massachusetts.

Esox vermiculatus, the mud pickerel, and *Amia calva*, the bowfin, harbor natural infections of *A. longa* in the vicinity of Ann Arbor, Michigan. The life history of this trematode has been experimentally determined in this laboratory.

Eggs from the terminal portion of the uterus of specimens of *A. longa* contained active, mature miracidia. When these eggs were fed to laboratory-raised *Ammicola limosa*, various intramolluscan stages developed. Full development of cercaria-producing rediae was attained at 21 days. Characteristic cystocercous cercariae began to emerge 42 days after infection. Cercariae fed to specimens of *E. vermiculatus* developed directly into egg-bearing worms 20 to 30 days later.

Cystocercous cercariae emerged from two of 13,500 *A. limosa* collected and these cercariae appeared to be morphologically identical with those obtained from experimental infections.

A detailed account of this life cycle with complete descriptions of intramolluscan stages and observations on the biology of *A. longa* is now in preparation and will appear elsewhere.

17. *Life History of a Strigeid Trematode, Hysteromorpha triloba (Rudolphi, 1819) Lutz, 1931*. ERNEST J. HUGHINS, South Dakota State College, Brookings.

In the fall of 1949, the writer obtained from 3,500-acre Spring Lake, near Savanna, Illinois, some black bullheads, *Ameiurus melas*, whose flesh was riddled with tiny white cysts. The incidence of infection was extremely high in the entire bullhead population and was leading to a serious decrease in the fishery value of the lake. The cysts were found to be strigeid metacercariae of the *Diplostomulum* type. At a secluded edge of the lake was a double-crested cormorant (*Phalacrocorax a. auritus*) rookery of approximately 300 birds. Autopsy of some of these birds yielded a few strigeid trematodes which were identified as *Hysteromorpha triloba*. Nestling birds were captured and fed heavily infected bullheads; upon autopsy, their intestines were almost occluded with many thousands of *H. triloba*.

Ciurea (Arch. roumaines Pathol. exper. Microbiol. 3: 277) described the metacercaria of *H. triloba* in Roumania. Lutz (Mem. Osw. Cruz 25: 333), unaware of Ciurea's work, published on the life history of *H. triloba*, but his descriptions did not agree with those of Ciurea. Olivier (J. Parasitol. 26(6): 447) doubted the validity of Lutz's work. The writer has worked out the complete life history of *H. triloba* in the laboratory and has shown Lutz' work to be totally erroneous.

The unembryonated eggs hatch in 7 days at 37° C. Miracidia penetrate *Gyraulus hirsutus*, which shed cercariae in 15 days at high summer temperatures. Cercariae readily penetrate

black bullheads and bluntnose minnows, *Hyborhynchus notatus*, but will not develop in guppies, *Lebistes*. Metacercariae reach full development in about 12 weeks. They develop into egg-laying adults 3 days after reaching gut of cormorant.

Detailed anatomical, developmental, behavioral, and ecological studies were made, including first descriptions of sporocysts and cercariae and first complete description of miracidia.

This work was done at the University of Illinois and was supported in part by a National Institutes of Health Research Fellowship.

18. *Studies on the Life Histories of Maritrema obstipum* (Van Cleave and Mueller, 1932) and *Levinseniella* sp. (Trematoda: Microphallidae). F. J. ERGES, New York University.

The life histories of *Maritrema obstipum* and a species of *Levinseniella* are reported. Larval and adult stages of both species are described from natural and experimental infections. The cercariae of both species emerge from the snail, *Amnicola pilsbryi* Walker, 1906, penetrate and encyst in the aquatic isopod, *Asellus communis* Say, 1818, and experimentally reach sexual maturity in various birds and mammals. The adult of *M. obstipum* is usually found in the small intestine, and *Levinseniella* sp. localizes chiefly in the cecum.

The action of Cable and Kuns (1951) in suppressing the family Maritreematidae Baer, 1943 is supported by additional experimental evidence. The genus *Pseudospelotrema* Yamaguti, 1939 is suppressed as a synonym of *Maritrema* Nicoll, 1909, and the species *Pseudospelotrema japonicum*, *P. uriae*, and *P. cincli* are transferred to *Maritrema*. The genus *Maritreminoides* Rankin, 1939 is restored to contain *Pseudospelotrema nettae* and *P. amnospizae*. *Pseudospelotrema obstipum* and *P. medium* are returned to the genus *Maritrema*.

Maritrema obstipum is differentiated from *M. medium* on both morphological and bionomic grounds. The cercaria of *M. obstipum* is shown to differ from the Ubiquita type described for other members of the family Microphallidae, and is regarded as similar to the cercariae of *M. rhodanicum* described by Carrère (1936) and *M. caridinae* described by Shibue (1951).

The genus *Gynaecotyla* Yamaguti, 1939 (Syn. *Cornucopula* Rankin, 1939) is suppressed as a synonym of *Microphalloides* Yoshida, 1938. *Gynaecotyla adunca*, *G. jägerskiöldi*, *G. similimus*, *G. squatoralae*, and *G. nassicola* are transferred to *Microphalloides*.

19. *Studies on the Encystation of Larval Digenetic Trematodes*. FRANCIS J. KRUIDENIER, The University of Illinois.

In commonly familiar life histories of various digenetic trematodes the cercaria normally does not establish relationship with a true host. In these instances the cercaria encysts variously on general or specific animate or inanimate objects. Such cercariae furnish the entire complex of encapsulating materials. Early studies provide a general concept of the process of encystation which has, possibly, become the "classic" explanation. Namely, cyst walls are formed by the extrusion of glandular materials from cercariae upon their contact with and adhesion to appropriate objects. Such cercariae are free-swimming and have, of course, emerged from the molluscan host.

Various investigations of fasciolids, although not in specific agreement and therefore confusing in detail, indicate that certain cystogenous materials are deposited prior to the emergence of cercariae from snail hosts. Present studies of the cercariae of *Fasciola hepatica* confirm and amplify this concept. Materials which form the outer cyst wall of the metacercaria of this species are secreted and discharged by cercariae in *redia*. The substances undergo maturational changes prior to the emergence of cercariae from the snail host and, in effect, form a pliable, loosely coherent, cuticula-like cyst wall which almost completely surrounds the free-swimming organism. The covering is deficient over the suckers.

Further investigations indicate that a similar phenomenon occurs in diverse species of digenetic trematodes. Thus it must be considered a common method of encystation rather than an exception.

20. *Experimental Infections of Rhesus Monkeys with Schistosomatium douthitti* (Cort). I. G. KAGAN, University of Pennsylvania.

Sexually mature or young developing male and female worms of *Schistosomatium douthitti* were recovered from the venules of the liver or intestinal veins of nine rhesus monkeys, *Macaca mulata* exposed to the cercariae of *S. douthitti*. These animals were exposed to from 2500-45,000 cercariae and necropsied 10-25 days later. Five monkeys exposed to 6000-10,000 cercariae of *S. douthitti* and necropsied 30-96 days after exposure were negative.

The recovery of dead and dying worms from monkeys necropsied 10-25 days after exposure suggests that resistance to infection in this host may begin as early as the second week after

exposure. The recovery of no worms in 5 animals necropsied 30-96 days after exposure suggests that the infection is completely terminated in 3-4 weeks.

Monkeys exposed to the cercariae of *S. douthitti* develop a schistosome dermatitis very similar to the dermatitis reported for man. In the monkey the rash disappears in 5-7 days. In animals previously exposed to the cercariae of *S. douthitti* the dermatitis which develops after second exposure was not as intense as the dermatitis which developed in animals exposed for the first time.

Monkeys exposed to cercariae of *S. douthitti* are resistant to a second exposure of *S. douthitti*; however, a previous exposure of *S. douthitti* does not protect an animal from infection with *S. mansoni*. From the pathology of the lungs at necropsy it appears that in resistant animals cercariae do not reach the lungs. This paper has been accepted for publication in the Journal of Infectious Diseases.

21. *Bisexual Reproduction in the Mother Sporocyst of Paragonimus kellicotti (Trematoda)*. ARTHUR E. WOODHEAD, Department of Zoology, University of Michigan, Ann Arbor.

In the trematode *Paragonimus kellicotti* Ward, bisexual reproduction is the method followed by the mother sporocyst in producing the next generation. The mature mother sporocyst is a hermaphroditic adult and the generation a true generation, since reduction occurs in both the male and female germ cells. The typical pattern of spermatogenesis is followed and the spermatids metamorphose into small oval cells, each with a condensed nucleolus and very little cytoplasm. At the metaphase of the primary spermatocyte 4-parted tetrads are present.

The development of the stalked oögonium begins with increase in size followed by detachment from the germinal tissue. The nucleus divides and the new cell (micromere) becomes a cap-cell (investing cell). A sperm cell is taken in, making a cluster of three cells. The cap-cell divides to form a cellular cover. The oöcyte and sperm cell attach to the new cover. When 6-8 cells are present in the cover, the sperm cell is detached and round, the primary oöcyte forms a metaphase plate and sets off polar body number one. This is a large cell which divides into two cells (polar bodies, two and three) which later grow into large cells. Polar body number two is set off and also becomes a nurse cell. When 40-80 nurse cells have been formed from the polar bodies and the mass (an egg) becomes oval, then the male and female chromosomes meet on a spindle and embryological development of the mother redia begins.

(Paper accepted to appear in Jan. or April issue of Trans. Amer. Micro. Soc.)

22. *Observations Made by the World Health Organization Schistosomiasis Team in the Philippines*. DONALD B. McMULLEN, Army Medical Service Graduate School; BENGT HUBENDICK, Naturhistoriska Riksmuseet (Stockholm); T. P. PESIGAN, Department of Health (Manila); and PAUL BIERSTEIN, W. H. O. Western Pacific Regional Office.

Schistosomiasis japonica in the Philippines is primarily a rural disease, with 100,000 to 200,000 cases in 12 endemic areas. Agricultural practices contribute to the seriousness of the problem. Eastern Leyte, Panguil Bay and Compostela endemic areas appear to be the most important and in that order. In most areas molluscacides are impractical under existing conditions. For this reason an attempt should be made to study the biology and ecology of the intermediate host in the hope of discovering means of environmental control. For the first time *O. quadrasi* was found in stream origins in virgin forest. These habitats occur on alluvial plains suitable for rice culture. When the forest is cleared for farming many of the snail habitats are left more or less undisturbed. Some evidence was obtained that indicated that *S. japonicum* is also present in the forested areas and that the commonly accepted relationship between the disease, man and rice farming is a comparatively recent development.

23. *Nocturnal Emergence of Schistosoma mansoni Cercariae from Australorbis glabratus by Inversion of Light Cycle and Practical Application*. GEORGE W. LUTTERMOSER, National Institutes of Health, Bethesda 14, Maryland.

The pattern of emergence of *Schistosoma mansoni* cercariae from *Australorbis glabratus* is known to be diurnal under normal conditions. The pattern of emergence of cercariae from infected snails isolated individually in beakers and in an incubator at constant 30°C. and light cycle from 9 p.m. to 9 a.m. was nocturnal but this became diurnal when the light cycle was 9 a.m. to 9 p.m. It was found, however, that usually a low percentage of the cercariae escaped during the period of darkness. It can be recorded, incidentally, that in other tests when snails were isolated in fresh water or in water in which they had been living for 2 weeks, the emergence of cercariae appeared to be stimulated.

The common practice of placing infected snails in light at 9 a.m. and then harvesting cercariae at 2 p.m. was not convenient for exposure of large numbers of animals the same after-

noon. The evening before exposure, groups of snails were isolated and were placed in a light-proof box equipped with a blower or in a small forced-draft oven (Hotpack Company). The fan and fluorescent lamps installed in each of these cabinets were turned on by an electric time switch at 3 a.m. and a temperature range of from 30° to 32° was maintained. By 9 a.m. cercariae were harvested and were found to be as infective as those obtained by the usual procedure.

24. *Pulmonary Manifestations of Single Sex Schistosoma mansoni Infections in Mice.* WILLIAM B. DEWITT, National Institutes of Health, Bethesda, Maryland.

In mice infected with *Schistosoma mansoni*, marked differences in distribution of the worms and resulting pathology occurred in single and mixed sex infections. The mice that received male cercariae only were found to have large numbers of mature worms in their lungs. Some of the worms were walled off by local tissue reaction and were partially degenerated; others were free in the tissue and were alive. Lung damage was extensive. In the case of female *S. mansoni* infections, however, the lungs appeared essentially normal. An occasional worm could be found, but as usual in exclusively female infections the worms were very small, immature specimens. In mice with mixed sex infections, no worms were found in the lungs.

25. *Penetration of Definitive Host Skin by Cercariae of Schistosoma mansoni.* M. A. STIREWALT, Naval Medical Research Institute, Bethesda, Maryland.

The pattern of penetration has been studied in the unshaved, unbroken skin and mucous membrane of the mouse. Direct observation of cercarial penetration and study of fixed and stained sections of invaded tail, belly and ear skin, and tongue of adult mice, and body and tail skin of one-day old mice indicated that, wherever possible, these cercariae entered by breaks in the surface, especially by way of hair follicles.

Cercarial penetration of skin was influenced by many factors among which were: age of mouse host, previous recent invasion, type and temperature of water used for cercarial suspensions, age of cercariae, region of skin penetrated. Mouse strain, mouse sex, cercarial "sex," and prophylaxis with immune serum had no effect on the number of cercariae which penetrated.

26. *Host-Parasite Relationships of Schistosoma mansoni.* J. H. THOMPSON, JR., Section of Clinical Pathology, Mayo Clinic, Rochester, Minnesota.

This study deals with resistance to reinfection in common laboratory animals to *Schistosoma mansoni*, and an experimental investigation of the behavior of the parasite in different hosts with respect to the problem of immunity. In addition to experiments devoted to host resistance and susceptibility and resistance to reinfection in the albino mouse, golden hamster, albino rat, and guinea pig, one highly susceptible host, the albino mouse, and one highly resistant host, the albino rat, was similarly studied with and without the presence of the spleen.

From the experimental data recorded in this study certain conclusions are drawn which broaden the knowledge of host-parasite relationships of *Schistosoma mansoni*, and extend the field of immunity studies relative to this important human parasite.

27. *Observations on Immunity to Superinfection with Schistosoma mansoni and S. haematobium in Monkeys.* HENRY E. MELENEY AND DONALD V. MOORE, New York University College of Medicine.

The observations reported here were made on four monkeys, one exposed repeatedly to bisexual infections of *Schistosoma mansoni*; one exposed and re-exposed to bisexual infection of *S. haematobium*, then exposed to bisexual infection of *S. mansoni*; one exposed to female cercariae and later to male cercariae and one exposed to male cercariae and later to female cercariae of *S. mansoni*.

The first monkey (*Pithecus mordax*) demonstrated spontaneous cessation of egg passage following active infection with *S. mansoni*, and complete immunity to repeated reinfections with the same species of worm, although cercarial penetration was demonstrated.

The second monkey (*Macaca mulatta*) demonstrated spontaneous cessation of egg passage following active infection with *S. haematobium*; complete immunity to one reinfection with the same species of worm, and lack of complete immunity to subsequent infection with *S. mansoni*.

The third and fourth monkeys (*M. mulatta*), each infected with one sex of *S. mansoni* and subsequently exposed to cercariae of the opposite sex, demonstrated that a unisexual infection of either sex did not prevent the development of the opposite sex to maturity within the normal period of maturation. (Supported by a grant (E-27) from the National Institutes of Health) (Accepted for publication in *Experimental Parasitology*).

28. *Studies on the Nervous System of Postharmostomum helices* (Leidy, 1847) Robinson, 1949, (Trematoda: Brachylaematiidae). M. J. ULMER, Iowa State College.

Modified gold chloride impregnation techniques on sections of young laboratory-reared adult worms demonstrate, in addition to the major nerve trunks common to most digenetic trematodes, the presence of additional commissures, particularly in the pharyngeal region. Transverse pre-acetabular commissures, considered to be lacking by some investigators dealing with this group, appear to be well-developed. Of the four pairs of anterior and posterior nerve trunks, the dorsal group is least readily seen. Details to be published in the Transactions of the American Microscopical Society.

29. *Studies on schistosomiasis. IX. Studies on Some Ointments for Protection Against Schistosomiasis Mansoni.* M. G. RADKE, G. W. HUNTER III, A. P. MOON, D. E. POTTS, AND J. S. WILLIAMS, Fourth Army Area Medical Laboratory, B.A.M.C., Fort Sam Houston, Texas.

Compounds have been tested as potential protective ointments against the cercariae of *Schistosoma mansoni*. The chemicals tested were incorporated in olive oil and petrolatum to give the proper consistency for application of the ointment; in one case an emulsion of 5 per cent aqueous n-butylacetanilide and Tween 80 was utilized.

Mice (with shaved backs) which were protected by a single application of ointment under test were exposed to 100 cercariae of *S. mansoni* for 30 minutes. These were sacrificed and perfused for schistosomes in six to eight weeks following exposure. Ninety-seven unprotected mice served as controls from which an average of 49 worms per mouse were recovered; 25 per cent olive oil, 50 per cent petrolatum, and one per cent Tween 80 were applied to 11, 12, and 15 mice respectively and an average of 51, 47, and 44 worms per mouse respectively were recovered. The chemicals screened were as follows: 5 per cent aqueous n-butylacetanilide—17 mice, 4 worms per mouse; 1 per cent n-butylacetanilide ointment—17 mice, 2 worms per mouse; 5 per cent n-butylacetanilide ointment—19 mice, none; 10 per cent Netotran—12 mice, 19 worms per mouse; 20 per cent Neotran—14 mice, 14 worms per mouse; 20 per cent Ovotran—14 mice, 4 worms per mouse. The 5 per cent n-butylacetanilide ointment was the only one giving complete protection.

Pilot experiments with Covicone cream (Abbott), copper oleate (Eimer and Amend) and other ointments are in progress. These and closely related compounds will be tested subsequently.

30. *The Influence of pH, Temperature and Light on the Emergence of the Cercariae of Schistosoma japonicum from Oncomelania nosophora.* ADALINE GUMBLE, Y. OTORI, L. S. RITCHIE, AND G. W. HUNTER, III, 406th Medical General Laboratory, Tokyo, Japan.

The effect of several environmental factors on the emergence of *Schistosoma japonicum* cercariae has been studied. The variable results obtained may have been due to inherent differences of the several strains of *Oncomelania* used, or possibly strain differences of the parasite.

The effect of pH on emergence of *S. japonicum* cercariae was determined for naturally infected *Oncomelania nosophora*. The water was buffered for pH intervals of 6.0, 6.8, 7.2, 7.6 and 8.0. Within this range, the cercariae emerged freely, but at 6.0 and 8.0 (particularly the latter) there was evidence of suppression.

Normal temperatures of 12–28° C. did not affect the emergence of cercariae of *S. japonicum*. Higher temperatures caused distortion of the larvae. At 8° C. or lower shedding was suppressed. Animal exposures will be required to determine if their infectivity was affected.

Cercarial emergence was greatly reduced by the absence of light. Infected snails in darkness under otherwise favorable conditions of shedding gave off approximately one-third as many cercariae as controls over an 11 day period. Subsequently, when experimental snails were transferred from darkness to light, their shedding sharply increased equal to that of controls.

31. *The Biology of a Marine Dermatitis-Producing Schistosome Cercaria from Batillaria minima* (Gmelin). LAWRENCE R. PENNER, University of Connecticut, Storrs.

Experimental schistosome dermatitis has been produced with a species of avian schistosome cercaria which emerges from the black horn shell, *Batillaria minima* (Gmelin), collected mostly from somewhat sandy, shallow, and usually grassless bays off Longboat Key between Bradenton and Sarasota, Florida. This dermatitis simulates naturally acquired cases of what has been termed Gulf Coast Itch and correlates with the distribution of infected snail hosts where water-birds wade and defecate. A number of chickens exposed to the cercariae were negative upon examination but two of three young pigeons exposed were found infected. Adults obtained experimentally compare favorably with adults present in the portal system of the royal tern, *Thalasseus maximus*, collected from this area and from near Englewood, Florida. The experi-

mentally and naturally collected schistosome adults have been identified as *Ornithobilharzia canaliculata* (Rudolphi, 1819) Odhner, 1912. This was the first species of schistosome worm to be discovered, having been reported originally as *Distoma canaliculatum* from *Sterna* sp. by Rudolphi in 1819.

32. *The Red-Breasted Merganser as a Natural Avian Host of the Causative Agent of Clam Diggers' Itch.* LAWRENCE R. PENNER, University of Connecticut, Storrs.

Although laboratory infections of a large number of species of birds, including chickens of any age, can be easily produced by exposures of these birds to *Cercaria variglandis* Miller and Northrup, 1926, scant data are available regarding natural hosts of the adults. In the belief that the habits and migrations of the red-breasted merganser, *Mergus serrator*, fitted the picture better than those of any other winter resident along the Connecticut shoreline, and because the habits and peak cercarial infections in the snail host, *Nassa obsoleta*, were also in agreement with this premise, an attempt was made to examine some of these birds. Five were collected during November and December, 1952, all of which were found infected with adult schistosomes; these compared favorably with experimentally produced adults in pigeons and chickens.

A study of 22 specimens collected from *Mergus serrator*, a study of a series of several hundred experimentally obtained worms of ages up to and over a year, and a study of the type materials of *Microbilharzia chapini* Price, 1929, add convincing data that *Microbilharzia* Price, 1929, should be considered a synonym of *Austrobilharzia* Johnston, 1917. The name of this schistosome should now be *Austrobilharzia variglandis* (Miller and Northrup, 1926).

Worm eggs shed from mergansers during the winter months are likely responsible for a higher incidence of mature infections in the snail host during the late winter and early spring, resulting in more reports of clam diggers' itch during this time of year.

Studies on the role of the merganser as an important natural host are continuing.

33. *Experimental Infections of Avian Hosts with Cercaria littorinalinae Penner, 1950.* LAWRENCE R. PENNER, University of Connecticut, Storrs, and the Biological Research Institute, San Diego, California.

All species of birds, except ducks, exposed during the spring of 1950 to *Cercaria littorinalinae* were susceptible to infection and produced mature adult schistosomes when the size of the bird used would allow it. Among the hosts successfully infected were the brandt cormorant, black crowned night heron, western gull, budgerigar parakeet, Australian zebra finch, domestic pigeon, red jungle fowl, and linnnet. Some species of birds were severely affected and died as a result of exposure. Cercariae were obtained from *Littorina planaxis*, a common snail host for this species in the San Diego region.

The Brandt cormorant and the western gull are probably the best natural hosts of the worm in this area. The adult worms belong to the genus *Austrobilharzia* which has been emended to include the members of the genus *Microbilharzia*. A forthcoming paper will give a complete description of the adult worms and other pertinent data.

34. *The Life History of Entosiphonus thompsoni Sinitzin, 1931 (Trematoda: Brachylaemidae).* JOHN B. VILLELLA, University of Michigan.

Recent studies in this laboratory show that the small land snail, *Retinella indentata*, serves as an experimental first intermediate host in the life history of *Entosiphonus thompsoni*.

Adults of this brachylaemid were recovered from the small intestine of the short-tailed shrew, *Blarina brevicauda*. Of 85 shrews collected in the vicinity of Ann Arbor, Mich., 44 harbored mature and immature forms of the parasite. A few worms were also recovered from the small intestine of the white-footed mouse, *Peromyscus leucopus noveboracensis*.

Metacercariae were obtained from various species of land snails collected in areas from which shrews were trapped. Embryonated eggs from adult flukes were fed to laboratory-reared specimens of *Deroceras laeve*, *Retinella indentata*, *Succinea ovalis*, *Ventridens ligera*, and *Zonitoides nitidus*. Branching sporocysts developed only in *R. indentata*. Daughter sporocysts were discharged from mother sporocysts in approximately 40-45 days after exposure of the snail to eggs. Under laboratory conditions, during the months of October, November and December, cercariae were shed 70 days from the date snails were exposed to eggs.

Cercariae developed to metacercariae in several second intermediate snail hosts, notably *V. ligera*, *R. indentata*, *D. laeve*, and *S. ovalis*. Metacercariae were fed to hamsters, mice, rats, and rabbits. Sexually mature worms were obtained from the small intestine of laboratory *Peromyscus* three weeks after feeding metacercariae to this host. Embryonated eggs appeared in the feces 15 days following infection of *Peromyscus* with metacercariae. An account of the intramolluscan stages of *E. thompsoni* will be made in another report.

35. *The Life History of Ectosiphonous rhomboideus Sinitsin, 1931 (Trematoda: Brachylae-matidae) from the Short-tailed Shrew, Blarina brevicauda.* JOHN B. VILLELLA, University of Michigan.

Sinitsin (1931) described a brachylaemid metacercaria, *Ectosiphonous rhomboideus*, from the land snail *Gastrodonta ligera* (= *Ventridens ligera*). Metacercariae, the same as described by Sinitsin (1931), when fed to golden hamsters in this laboratory, developed into *Brachylaima rhomboideus*. This adult, which normally inhabits the small intestine of the short-tailed shrew, was described by Mason (1953) as *Brachylaima dolichodirus*. The latter name therefore, becomes a synonym of *B. rhomboideus* (Sinitsin, 1931).

V. ligera serves as a first intermediate host in Michigan for this brachylaemid. Embryonated eggs from adult worms were fed to laboratory-reared *V. ligera*, *Deroceras laeve*, *Mesodon thyroideus*, and *Retinella indentata*. Branching sporocysts developed only in *V. ligera*. Daughter sporocysts were discharged from mother sporocysts in 28-30 days after snails were infected with eggs. Mature cercariae were shed from daughter sporocysts 52-60 days after snails had been exposed to eggs. The mature cercaria of *B. rhomboideus* has ten pairs of flame cells.

Cercariae, in the slime secreted by infected snails, were transferred to suitable second intermediate hosts and developed to infective metacercariae in 75-90 days. Metacercariae developed in the salivary glands, kidney, and pedal sinus of *V. ligera*. Cercariae developed to metacercariae in several other species of land snails.

Feeding experiments were carried out with white rats, mice, rabbits, hamsters, and chicks. Sexually mature adults of *B. rhomboideus* were recovered from the small intestine of the hamster 21 days after metacercariae, 75-90 days old, were fed to this host. Adults were never recovered after 21 days. The infectivity in the hamster was low.

The details of the morphology of intramolluscan stages of this brachylaemid will be made in a future report.

36. *Life Cycle of Paramacroderoides echinus Venard, 1941, a Parasite of the Florida Gar, Lepisosteus platyrhincus.* RHODES B. HOLLIMAN AND W. HENRY LEIGH, University of Miami.

Helisoma normalis duryi, a common pulmonate snail in the canals of South Florida, has a high incidence of infection with a large xiphidiocercaria which penetrates and encysts in small fishes, usually *Gambusia affinis holbrooki*. In 23 days the metacercariae are infective for the Florida gar, *Lepisosteus platyrhincus*, which serves as a natural definitive host. The trematode becomes sexually mature in the posterior part of the gar's intestine as early as nine days after infestation at summer temperature.

Eggs of the trematode are embryonated when deposited and hatch only after ingestion by the snail host. Cercariae, produced by orange sporocysts, are unique in that four penetration glands occur on the right side and five occur on the left. Other species of fish penetrated by this cercaria are *Jordanella floridae*, *Heterandria formosa*, *Mollienesia latipinna* and *Fundulus* sp. The frog, *Rana sphenoccephala*, is also readily penetrated by the cercaria.

Twenty-five of sixty-three gar examined were naturally infected with *P. echinus*, although no fish contained more than seven specimens.

37. *Notes on the Life History of Opisthorchis tonkai Wallace and Penner, 1939 (Trematoda: Opisthorchiidae).* E. I. SILLMAN, University of Michigan.

Opisthorchis tonkai was described by Wallace and Penner (1939, J. Parasitol. 25: 437) from muskrats taken at Tonka Bay, Lake Minnetonka, Minnesota. They also reported that metacercariae from the minnow, *Notropis deliciosus stramineus*, taken from Lake Francis, Isanti County, Minnesota, when fed to various laboratory animals, developed into flukes identical with those recovered from the muskrats.

Centrarchid fish, chiefly *Lepomis gibbosus*, and cyprinids, chiefly *Hyborhynchus notatus*, taken from the Huron River in the vicinity of Ann Arbor, Michigan, were found to harbor numerous opisthorchioid metacercariae contained within oval, clear, thin-walled cysts. Both cysts and enclosed metacercariae closely resembled those described for *Opisthorchis felinus*. Infected fish were fed to three muskrats, thirteen cats, and five white rats. One to four specimens of *O. tonkai* were recovered from one muskrat, two cats, and one rat. The worms from the muskrat were two to two and a half times larger than those recovered from the cats and rat. They were also larger than those described by Wallace and Penner from muskrats, and those taken by Edwards (1949, J. Parasitol. 35: 547) from a muskrat in New York State. In all other respects the worms were morphologically identical with those described as *O. tonkai*.

Embryonated eggs from the muskrat specimens were fed to 30 young laboratory-raised *Ammicola limosa*. Seven weeks later, pleurolophocercous cercariae were emerging from two of the snails. As many as 50 cercariae were liberated each day from one of the snails.

38. *Microtus pennsylvanicus pennsylvanicus*, a New Final Host for *Opisthorchis tonkai* Wallace and Penner, 1939 (Trematoda: Opisthorchiidae). E. I. SILLMAN, University of Michigan.

Opisthorchis tonkai has been described from naturally-infected muskrats in Minnesota by Wallace and Penner (1939, J. Parasitol. 25: 437) and New York by Edwards (1949, J. Parasitol. 35: 547). Two surveys of muskrats carried out in Michigan have failed to reveal its presence there in natural infections. A report of unidentified "bile-duct trematodes" from a muskrat was found in records of animal autopsies performed at the Department of Zoology of the University of Michigan, but the material is unavailable for further examination.

Two hundred and fifty *Microtus p. pennsylvanicus* taken in the vicinity of Ann Arbor, Michigan were autopsied. Eight specimens of *O. tonkai* were found in four voles from one area. They measured from 4.2 to 5.2 mm. in length, and were identical in every respect with worms described by Wallace and Penner and those obtained by Edwards.

Fish from the nearby river were not found to harbor opisthorchioid metacercariae. Few molluscs occurred in the area and these were not found to be parasitized with opisthorchioids.

This appears to be the first record of infection of voles with an opisthorchid trematode.

39. *Studies on the Life History of Posthodiplostomum minimum* (MacCallum, 1921). JOSEPH H. MILLER, New York University.

Physa heterostrophia, from a pond in the New York Botanical Gardens, shed longifurcate monostome cercariae which penetrated the sunfish *Lepomis gibbosus* and *Lepomis megalotus*, migrated via the circulatory system to visceral organs, and developed to infective metacercariae in thirty days. The metacercariae were identical with the type specimens of *Neascus van cleavei* (Agersborg, 1926). Metacercariae fed to one to four-day old chicks resulted in the recovery after 32 hours of adult strigeids identical with the type specimens of *Posthodiplostomum minimum*.

Previous to this study, two species of cercariae, *Cercaria multicellulata* and *Cercaria louisiana*, have been reported as the cercarial stage of *Posthodiplostomum minimum*. The cercariae used in the present study can be differentiated morphologically from both *Cercaria multicellulata* and *Cercaria louisiana* on the basis of the following characteristics: the absence of an anteriorly directed tuft of spines at the oral opening, body spines posterior to an oral crown of spines limited to ten definite rows extending to the posterior margin of the body, the presence of a pair of posteriorly directed body setae emerging laterally at the level of the most posterior pair of penetration glands, setae on the tail stem confined to three lateral groups, five pairs of caudal bodies present in the tail stem and the openings of the two excretory ducts on the proximal anterior surfaces of the furcae.

40. *The Entrance of the Cercaria of Echinoparyphium flexum* (Linton) Dietz, 1910, into Tadpole Kidneys. HAIG H. NAJARIAN, University of Michigan.

The metacercaria of *Echinoparyphium flexum* is found in nature in the kidneys of both tadpoles and adult frogs. Laboratory-reared adult frogs fail to become infected when exposed to the cercariae. Laboratory-reared tadpoles of the same species are easily infected. It is assumed that the infection of amphibians in nature takes place during the tadpole stage and the cysts are carried through metamorphosis into the adult stage.

Cercariae of *E. flexum*, when placed in a small dish with tadpoles of *Rana pipiens*, crawl over the entire surface and in a few minutes aggregate around the cloacal opening. Within ten minutes the cloaca has a solid plug of cercariae. Tadpoles exposed to cercariae for 5, 10, 20, 45, 60, and 90 minutes, respectively, were fixed and sectioned serially, in order to determine the path taken by the cercariae to the kidney.

The cercariae enter the cloaca, loose their tails and enter the mesonephric ducts. Ascending the ducts, they elongate considerably and then enter the kidney tubules where they encyst. Encystment takes place as early as 20 minutes after exposure. Cercariae occur in the gill chambers and in the coils of the gut, but in both cases encystment does not take place.

41. *A Partially-Twinned Daughter Redia*. HAIG H. NAJARIAN, University of Michigan.

From a naturally infected specimen of *Lymnaea palustris*, from which cercariae of *Echinoparyphium flexum* (Linton) Dietz, 1910, were emerging, a large "partially-twinned" daughter redia was recovered. The redia had a single pharynx, 39 by 41 microns and a single birth pore on the left side. No collar was present. Shortly posterior to the level of the birth pore, the redia was split into two bodies, both the same length and width, viz., 186 by 52 microns. Each body had a pair of lateral appendages directed medially. One body had 22 mature cercariae;

the other had 24. No gut was discernible. Since twinning began shortly posterior to the birth pore, it is probable that only a single gut was present. Attempts to find similar rediae in the same snail and in hundreds of snails subsequently examined were unsuccessful.

42. *An Ancyloid Snail, Ferrissia novangliae, a First Intermediate Host for Trematodes.* R. JAY SMITH, University of Michigan.

During the months April through October 1951-52, intra-molluscan stages of six species of trematodes were found to be infecting ancyloid snails, *Ferrissia novangliae* Walker, 1908. This is believed to be the first report of trematode infections in North American ancylics.

The following percentages represent the infections found in over 10,000 of these snails collected from one marsh-pond near Chelsea, in southeastern Michigan: furcocercous strigeid cercariae, two species, each six percent; furcocercous spirorchid cercaria, six percent; echinostome cercaria, 19 percent; xiphidiocercaria, three percent; amphistome cercaria, as high as 60 percent.

Ferrissia novangliae rarely exceeds three mm. in length. They were abundant in this and other nearby marsh-ponds. Collecting these fresh-water limpets was facilitated by removing them with a camel's-hair brush from the sides of enamelled pans which were filled with pond water and bottom vegetation.

Several other species of ancylics, *F. fusca*, *F. parallela* and *F. rivularis* were collected from other areas around Ann Arbor, Michigan. These were negative for trematode infections.

43. *Fasciolopsis buski (Lankester) in Central Thailand.* ELVIO H. SADUN, Division of International Health, U. S. Public Health Service.

Infection with *Fasciolopsis buski* in Thailand was believed to be rare. A spot check was carried out in various areas in Central Thailand where water caltrops (*Trapa bicornis*) are cultivated, to determine whether or not endemic centers of fasciolopsiasis existed.

A total of 784 males and 779 females chosen at random in three Provinces in the neighborhood of water caltrop plantations, was examined. By simple smear examination 208 persons, equal to 13 per cent were found infected with the intestinal fluke. No significant difference of the percentage infection was encountered between the two sexes. The distribution of this helminth in the various age groups was as follows: 1 to 4 years: 10%, 5 to 9 years: 15%, 10 to 14 years: 15%, 15 years and over: 8%. Stoll counts were done on 125 individuals. The average egg count of the persons harboring *Fasciolopsis buski* was 5,100 eggs per ml. The intensity in the various age groups was as follows: 1 to 4 years: 1,700; 5 to 9 years: 4,500; 10 to 14 years: 6,600; 15 years and over: 2,600. The most severe infection encountered was in a girl 15 years old who passed 88,000 eggs per ml. of feces. Following hospitalization the patient vomited 29 adult flukes and passed 6 in her stools. In spite of supporting measures the patient developed general anascarca and died. At autopsy 466 flukes were found in the lumen of the small intestine. Congestion of the lungs, spleen, kidneys and liver, and degeneration of the liver cells with fat vacuoles in the cytoplasm were seen by pathological examination.

44. *Preliminary Report on the Treatment of Opisthorchiasis with Aralen (Chloroquine diphosphate) in Korat Province (Northeast Thailand).* ELVIO H. SADUN, Division of International Health, U. S. Public Health Service, and CHAMNIAN CHARNMARNKITCH, Department of Health, Thai Ministry of Health.

A preliminary study was carried out to observe the effect of Aralen on infections with *Opisthorchis viverrini*.

A total of 15 soldiers, 21 to 22 years old, was selected for this study. On the day before treatment they were all positive for *Opisthorchis viverrini* by simple stool smear examination. Stoll counts were carried out just before treatment and every other day after the beginning of the treatment up to the end of the administration of Aralen. The number of eggs per ml. of formed feces varied from 1,200 to 26,800 with an average of 4,100 for the group. Each patient received 1 gm. of Aralen per day for the first 3 days and 0.5 gm. per day for the following 20 consecutive days, with a total of 13 gm. Egg counts were also done between 10 and 50 days after the termination of treatment.

At the end of the Aralen administration 6 out of 15 soldiers passed no *Opisthorchis* eggs with their stools, 7 had a lower egg count and 2 had a higher egg count than they had before treatment began.

Ten to twenty days after the termination of treatment out of 13 soldiers examined 7 were negative, 4 had a lower egg count and 2 had a higher egg count than previous to treatment. Forty to fifty days after the termination of treatment, out of 12 soldiers examined 3 were negative, 7 had a lower egg count and 2 had a higher egg count than on the day previous to treat-

ment. No toxic effects other than mild nausea, occasional loose stools and one case of temporary giddiness were observed.

Further studies in other areas are being carried out and will be reported elsewhere.

45. *A Method of Preparation of Whole Mounts of Miracidia and Cercariae*. J. STEGER HUNT, Zoology Department, University of Michigan.

A modification of the method of preparing whole amounts of free-living Protozoa by affixing them to slides by use of Carnoy's solution has been applied to similar preparations of miracidia and cercariae.

A drop of water containing a concentration of miracidia or cercariae is placed on a slide which has been smeared with Mayer's albumen fixative. Most of the water is permitted to evaporate; just before dryness is reached, a drop of Carnoy's solution (absolute alcohol 6 parts, glacial acetic 1 part, chloroform 3 parts) is placed on the organisms by use of a micropipette. As this solution dries, it is followed by a drop of absolute alcohol.

During these operations, it is advantageous to keep the organisms under observation with a stereobinocular microscope.

The material may be carried down the series of alcohols and through a staining process by dropping the liquids on the slide or Coplin jars may be used. A protective film can be placed on the material by flooding the slide with a thin layer of dilute nitrocellulose solution before it is passed into 70% alcohol. When 70% alcohol is reached, the material may remain for an indefinite period. Gorgoderid miracidia mounted in this manner and stored in Coplin jars of 70% alcohol for more than a year did not lose their stain receptivity.

This method has given uniformly good results with miracidia and with small and medium sized cercariae. By exercising care, satisfactory results have been obtained in a majority of cases with large gorgoderid cercariae.

46. *Bialovarium nocomis* n.g., n.sp. (Cestoda: Caryophyllaeidae) from the Hornyhead Chub, *Nocomis biguttatus* (Kirtland). JACOB H. FISCHTHAL, Harpur College, State University of New York, Endicott, New York.

A single specimen of an unsegmented tapeworm (Caryophyllaeidae), recovered from the small intestine of the hornyhead chub, *Nocomis biguttatus*, from Meadow creek, Barren County, Wisconsin, is described as *Bialovarium nocomis* n.g., n.sp. The diagnosis of the new genus is as follows: Caryophyllaeidae with poorly defined scolex, bearing a pair of shallow loculi. Cirrus opens into utero-vaginal canal before it reaches the surficial atrium. Ovary V-shaped and entirely medullary. Uterine coils extend only to lateral margins of cirrus sac, attaining a maximum longitudinal extent equivalent to one-fourth that of testicular field. Terminal excretory bladder present. Seminal vesicle not inclosed in cirrus sac. Post-ovarian vitellaria absent. Parasitic in Cyprinidae. Development unknown.

47. *Morphological Observations on Normal and Triradiated Taenia pisiformis from the Dog*. E. E. BYRD AND F. W. FIRE, University of Georgia.

During September, 1952, several taeniid tapeworms were removed from the small intestine of a collie dog. The dog came from South Dakota and was autopsied in Wilmington, North Carolina. When these tapeworms were examined in this laboratory, three of the specimens proved to be triradiated. From serial sections and whole mounts of the triradiated specimens, it was seen that both the scolices and the strobilae were involved in the radiate condition. The genital pores were irregularly alternated between the three rays. In one specimen, a segment was found in which a supernumerary proglottid was present. This proglottid possessed three genital pores although there were but two ovaries. Over 400 testes were counted in this one segment. The other specimens of the *Taenia* appeared to be normal.

48. *The Effects of Taenia taeniaeformis and Amphimerus pseudofelineus on the Liver as shown by Histochemical Techniques*. ROBERT M. LEWERT AND CHANG LING LEE, The University of Chicago.

Studies were made of the rat liver with two day to several month infections with *Taenia taeniaeformis*, and an infection of the cat with *Amphimerus pseudofelineus*. Frozen dried tissues were prepared and examined using various histochemical methods. These were selected to show alterations resulting in or associated with soluble substances not readily localized by other techniques and include observations on glycogen, glycoprotein, and phosphatase distribution. Changes in the basement membranes of the hepatic sinusoids were found indicating a depolymerization and increase in soluble glycoprotein associated with the presence of the early stages of

the strobilocercus. In the case of the *T. taeniaeformis* infections, these changes closely resemble those seen in newly regenerated liver. In the fluke infection changes are particularly evident close to the areas of hyperplastic bile duct epithelium. *Taenia taeniaeformis* extracts were tested for collagenase activity using the Azocoll technique. Only extracts prepared from young strobilocerci were found to be active. The findings suggest that substances produced by these worms act on the intercellular connective tissue elements in a manner similar to that previously reported by the authors as being associated with the penetration of the skin by various helminths.

49. *The Size Attained by Hymenolepis diminuta in Different Host Species.* CLARK P. READ AND MARIETTA VOGEL, University of California at Los Angeles.

In order to determine the possible effect of host species on the size of tapeworms, single worms of *Hymenolepis diminuta* were established in laboratory mice, albino rats, hooded rats and hamsters. All hosts were autopsied twenty days after infection. Volume of tapeworm tissue was used as the measure of size attained. The results showed clearly that there are significant differences in the size attained by *Hymenolepis diminuta* in different host species, and that this is related in a general way to the weight of the host. Average figures are as follows: mouse weight 27.6 grams, worm volume 0.28 ml.; hamster weight 123 worm volume 0.57; albino rats weight 377, worm volume 1.11; hooded rats weight 452, worm volume 1.33. (In press, Journal of Parasitology.)

50. *Evaluation of Field Procedures Related to Molluscicidal Applications.* J. R. C. FONSECA, J. E. WILLIAMS, R. E. FREYTAG AND L. S. RITCHIE, 406th Medical General Laboratory, Tokyo, Japan.

Several methods of applying molluscicides have been tested, including dusting, continuous-flow, impounded water, pressure spraying and sprinkling. Dusting with rotary dusters was impractical due to interference by wind. Continuous-flow, impounding and flooding possess the same general character, consisting essentially of adding a concentrated chemical solution at the water source. In the first case, the water flows at a depth of 4-6 inches, the sides and shoulders of the ditch being treated by splashing and pouring. Impounding is associated with short lengths of ditch and retention of the chemical for a period of time. Flooding utilizes such quantities of water as to secure effective heights of the chemical in the ditches and/or fields. The sprinkling method and efficiency have been well established (McMullen et al 1951 and Hunter et al 1952). With DN-1 continuous-flow showed a mortality of 90% (about 30 PPM or 0.2 gms./sq. ft.), impounding 90-99% (10-40 PPM or 0.2-0.4 gms./sq. ft., and flooding 57-100% (2.5-5 PPM or 0.2 gms./sq. ft.). The varied flooding results were chiefly due to extremes of snail distribution indicating the need of supplementing with sprinkling. The flooding types of procedure afford more thorough coverage than sprinkling, are suited for rock-lined and irregular ditches, and save labor; however, more chemical is required. Recent experiments have proven successful in replacing manual methods of sprinkling by utilizing gravity and a system of rubber hoses and sprayers on terraced hillsides. On flat areas, hand pumps locally employed for spraying crops might replace gravity. Large scale control may well require a variety of methods, although pressure spraying may in itself prove adequate. Local terrain, economic capability and available labor at present dictate the utilization of varied methods.

51. *Information Pertinent to Destruction of Eggs and Newly-hatched of Oncomelania nosophora by Molluscicides.* L. S. RITCHIE, Y. OTORI, J. R. C. FONSECA AND J. E. WILLIAMS, 406th Medical General Laboratory, Tokyo, Japan.

The possibility of effectively applying molluscicides against the eggs and newly-hatched of *Oncomelania nosophora* is currently under consideration. Available information, which implements such an undertaking, includes location of eggs and young in nature, the MLD of the molluscicides for these stages, seasonal duration of laying, and the period required for incubation.

Observations indicate that the young snail is aquatic for several weeks after hatching, assuring exposure to chemicals applied at irrigation head waters. Whether eggs are normally located in water or above the water line has not been determined. Under laboratory conditions, extensive laying and incubation have occurred on moist surfaces, but the preferential laying site in nature is not known. A matter of primary concern is whether the MLD for these stages is sufficiently low to compensate for wastage entailed in applying molluscicides directly into irrigation waters. Initial laboratory experiments indicate that dinitro-o-cyclohexylphenol in the formulation of DN-1 is highly effective at a concentration of 1 part (active ingredient) in 2 million with six-hour exposures. The newly-hatched snail is essentially without protection of shell and operculum for a period after hatching and, consequently, is highly vulnerable to

toxic agents. The eggs appear to be much more resistant. Evidence is available which indicates that egg-laying may occur at any time from early May to early August, varying for different spots even within the same area. These observations indicate the necessity of repeated chemical applications during the summer to assure exposure of all young snails. If all existing eggs as well as newly hatched could be destroyed by a single application, the incubation period would then serve to determine the application interval. It has been shown that incubation ranges from 12-35 days, with a majority of the eggs hatching after 18-24 days. This suggests a three-week application interval. If practical molluscacidal concentrations are inadequate to kill eggs, but prove effective against the newly-hatched, an alternative application interval might be one which is equal to the post-embryonic period required for the snail to develop a protective shell.

52. *Determination of the Relative Efficiencies of Molluscacides by Means of Field-plot Dilution Tests.* J. E. WILLIAMS, R. E. FREYTAG, J. R. C. FONSECA AND L. S. RITCHIE, 406th Medical General Laboratory, Tokyo, Japan.

About 25 molluscacides previously recognized as effective against *Oncomelania nosophora* (McMullen, 1952 and Hunter et al, 1952) were given field-plot dilution tests in 25 foot ditch sectors (100 sq. ft.) with quantites of active ingredients ranging from 0.6 to 0.0125 grams/sq. ft. Previously recommended amounts of sodium-pentachlorophenate (Santobrite and Dowicide G) and dinitro-o-cyclohexylphenol (DN-1) were confirmed.

Tests performed in 1953 were carried out during a rainless period, which probably accounted for the reduced efficiencies of the controls, Santobrite and DN-1, and may have resulted in less than maximum efficiency of the test chemicals. A few very promising molluscacides with the smallest amount of active ingredient affording at least a 90% kill, and the amount per sq. ft. suggested for field tests, are as follows:

Formulation	Active Ingredient	Gms./sq. ft.	Efficiency	Gms. for field tests
Santobrite	Sodium pentachlorophenate	.6*	89%	.4
DN-1	Dinitro-o-cyclohexylphenol	.2*	82%	.1
Dow CE 28	Dinitro-o-cyclohexylphenol	0.025	92%	.05
Dupont				
Weed Killer	Dinitro-o-sec-butylphenol	0.1	95%	.05
Dow CE 30	Dinitro-o-sec-butylphenol	0.025	96%	.05
Dow CE-27	Dinitro-isopropylphenol	0.025	94%	.05
Dow CE 29	Dinitro-phenylphenol	0.025	91%	.05
DN-2	Dinitro-o-cresol	0.1	92%	.1

* Largest quantity used.

53. *Studies on the Lone Star Tick. II. Preliminary Plot Tests of Potential Acaricides (Tickicides).* A. A. THERRIEN, G. W. HUNTER, III, A. P. MOON, A. L. ADAMS, D. E. POTTS, M. G. RADKE, J. S. WILLIAMS, AND J. E. WEBB, JR. Fourth Army Area Medical Laboratory, B. A. M. C., Port Sam Houston, Texas.

Five chemicals (Dieldrin, Aldrin, Lindane, DDT, Chlordane) were applied in varying dilutions to a series of 1/40 acre plots at Camp Bullis, Texas, in order to test their efficacy against the Lone Star tick. The criteria sought were: (1) rapid knockdown, and (2) residual effect. These chemicals were applied at the following rates: Dieldrin at $\frac{1}{4}$ lb., $\frac{1}{2}$ lb., and 1 lb. per acre; Aldrin at $\frac{1}{2}$ lb., $\frac{1}{2}$ lb., and 1 lb. per acre; Lindane at 1/20 lb., 1/10 lb., and $\frac{1}{4}$ lb. per acre; DDT at 1 lb., 3 lb., and 4 lb. per acre; Chlordane at 1 lb., 3 lb., and 4 lb. per acre. The last two chemicals had been tested previously and were used as a type of control together with a number of untreated plots.

One or two nine square foot quadrats were staked out in the area of greatest tick density in each plot and pretreatment counts were made on all plots. These were dusted; 24 and 48 hour counts were made, thereafter counts were made at 7 and 14 day intervals for 8 weeks. Dieldrin at 1 lb. per acre gave 97.9 per cent control in 24 hours and yielded the best residual effect at the end of four weeks, using Abbott's formula as a criteria of effectiveness.

54. *The Synthesis of Amino Acids from Ammonia in Hymenolepis diminuta.* JACK DAUGHERTY, The Rice Institute.

The failure of low protein diets to effect deleteriously the rat tapeworm, *H. diminuta*, establishes the existence of other sources of the nitrogen needed by the worm for growth and egg production. The diffusion of amino acids from the mucosa has been suggested as a source but

the apparent selective absorption of amino acids by the intestine argues against this. Experiments were undertaken to determine the availability to the worm of intestinal ammonia.

Living intact worms incubated for ninety minutes in Krebs-Ringer phosphate media (KRP) showed little difference from the controls. With the addition to the KRP of ammonium carbonate in amounts equivalent to that of the ammonia found in the rat intestine, there was an increase in the amino nitrogen. By chromatographical separation this was shown to be associated mainly with the alanine and glutamic acid fractions. The inclusion of pyruvic acid, oxalacetic acid, alpha-ketoglutaric acid, or glucose with the ammonia fortified KRP further increased the amino nitrogen. Alanine was uniformly the center of this increase. This contrasts with the results from rat liver slice studies where glutamic acid filled this role.

These facts demonstrate that the fixation of ammonia by intermediates of carbohydrate metabolism may constitute an important mechanism for the synthesis of protein in *H. diminuta*. The relation of alanine to pyruvic acid and the predominance of the former in ammonia fixation emphasize the anaerobic type of metabolism in *H. diminuta*.

55. *The Metabolism of Sulfur Amino Acids in Hymenolepis diminuta*. SEYMOUR GARSON AND JACK DAUGHERTY, The Rice Institute.

On the basis of bile duct ligation studies, to be described elsewhere, the unusually large amount of taurine found in *H. diminuta* appeared to have arisen as a result of the metabolism of the worm rather than from the bile constituents of the host. To test this hypothesis, S³⁵ labelled methionine was injected into 12 rats infected with *H. diminuta*. At intervals of 1, 4, 8, 18, 24, and 48 hours two animals were killed and the total activity of the worms and intestinal mucosa was measured on a windowless flow counter. The activity in the mucosa slowly rose to peak at 24 hours and gradually diminished thereafter. The activity in the worms steadily increased during the entire experiment. 24 hour samples of worm and mucosa were fractionated into the free amino acid and protein components and, following hydrolysis of the latter, the activity was traced to the constituent amino acids and related substances by chromatographic procedures. At 24 hours most of the total activity in the worm was in the protein fraction indicating a rapid incorporation of the methionine and its derivatives. In the free amino acid fraction of the worm nearly all the activity was found in cystine, glutathione, and taurine with very little activity associated with methionine. The protein hydrolysates of the 24 hour worm showed nearly all the activity associated with cystine whereas that of the mucosa showed the activity evenly divided between cystine and methionine. The analysis of 48 hour samples showed a further concentration of the worm activity in cystine. These observations support the hypothesis that methionine is actively degraded to taurine via cystine in *H. diminuta*.

56. *Vitamin B₁₂ and Desoxyribonucleic Acid Relationship in H. diminuta*. WILLIAM G. DACRES, WOLMAR NYBERG, AND JACK DAUGHERTY, The Rice Institute.

In the course of investigations on the metabolism of *H. diminuta*, studies were undertaken to determine the desoxyribonucleic acid (DNA) concentration and its metabolism in relation to vitamin B₁₂ and folic acid. By means of a microbiological assay using *Lactobacillus leichmanii* (ATCC 4797) the B₁₂ content of the worm was found to be in the range of 0.02 micrograms/gm. of dry worm. DNA content of erythrocytes, rat serum and worm was determined according to the method described by Ceriotti and found to be 450 to 750 micrograms/ml., 250 to 350 micrograms/ml., and 1000 to 1500 micrograms/gm., respectively. Rat serum and worm homogenate were incubated at 37° C. for 48 hours in the presence of DNA. Following incubation the reaction mixtures were analyzed for desoxyribosides and free purine and pyrimidine bases by paper chromatography using water saturated n-butanol as the solvent. The purine and pyrimidine spots were identified by ultraviolet light and the desoxyriboside spots were developed by the method of Buchanan. Rat serum and worm homogenate were found to be capable of degrading DNA to desoxyribosides. On the addition of vitamin B₁₂ and/or folic acid to the incubation mixtures the desoxyribosides were further broken down to purines and pyrimidines.

57. *Vitamin B₁₂ Uptake by Hymenolepis diminuta*. WOLMAR NYBERG, The Rice Institute and The University of Helsingfors (Introduced by JACK DAUGHERTY).

In the course of investigations on the intermediary metabolism of nucleic acids in *Hymenolepis diminuta* it became desirable to study the availability of vitamin B₁₂ to the worm.

Co⁶⁰ labelled B₁₂ (Merck and Co.) was given orally and intravenously to *H. diminuta* infected rats. After intervals of from 6 hours to 14 days the worms and certain tissues of the rats were prepared and their radioactivity determined with a well type crystal scintillation counter. No significant uptake of the radioactive B₁₂ by the worms obtained. In contrast, *H. diminuta* incubated for 24 hours in B₁₂ fortified Tyrode's glucose solution showed a significant uptake, which, however, did not increase with additional B₁₂. The addition of human or rat

gastric juice to the media allowed an increased uptake of $\text{Co}^{60}\text{-B}_{12}$ to an amount commensurate with the normal B_{12} content of the worm. The B_{12} content of the worm was determined by microbiological methods. The gastric juice presumably rendered the labelled B_{12} more metabolically available to the worm by establishing the conditions necessary for a more rapid turnover of B_{12} in the worm with the result that replacement by radioactive B_{12} occurred at a higher rate. Dialysis studies showed that in all cases the radioactive B_{12} taken up by the worm was metabolically bound and was not simply the result of free diffusion.

The discrepancy between the results of the *in vivo* and *in vitro* experiments cannot be explained at present. Investigations on this phase of the problem are now in progress.

58. *Auto-reinfection in White Mice Resulting from Infection by Hymenolepis nana*. D. HEYNEMAN, The Rice Institute.

A series of *Hymenolepis nana* infection studies in white mice has been undertaken to compare immune response induced by the direct cycle (egg infection) with that induced by the indirect cycle (cysticercoid infection). No direct immune reaction has been obtained from infection with cysticercoids, in contrast to the well-established immunity induced during the tissue phase resulting from egg-infection. The failure to induce any protective host reaction enables the eggs passed by a generation of worms infected via the indirect cycle to hatch in the lower portion of the ileum. Thus, by auto-infection, a massive infection of second generation worms derived from the direct cycle is produced. A series of previously uninfected six-week-old mice, each fed 30 cysticercoids developed in *Tribolium confusum*, on autopsy 21 days later harbored 1000–2000 very small immature worms in the lower ileum, in addition to the few remaining members of the parent generation. This auto-reinfection exists for the single generation only, for the cysticercoids developing in the villi stimulate an immune response which guards the host from continual internal multiplication of the tapeworm by the direct cycle. It is apparent that the tissue phase of the larvae developing from infection by eggs produces an immunity which prevents auto-reinfection (except in the event of starvation, vitamin deficiency, or illness of host).

59. *Cross-immune Protection Against Hymenolepis diminuta by H. nana in White Mice*. D. HEYNEMAN, The Rice Institute.

Hymenolepis diminuta has been shown by Chandler (1939, Amer. Jour. Hyg., 29 [Sec. D]: 105–114) to be ineffective in production of immunity in the white rat. He postulated that this is due to the lack of a tissue phase infection during growth of the worms in the rat intestine. The same appears to be true of the indirect cycle of *Hymenolepis nana* established in white mice as cysticercoids developed in *Tribolium confusum*. However, the direct cycle of *H. nana* produces an immunity as has been well substantiated by several workers. Three sets of 12 *H. nana*-immune mice with standing infections, infections 2 and 4 months old respectively (negative for eggs during that period), and 12 uninfected controls were each fed 10 cysticercoids of *H. diminuta* developed in *Tenebrio*. None of the *nana*-immune animals passed eggs of *H. diminuta*, whereas all of the controls did so. Upon autopsy 21 days later several of the experimental mice possessed one or two dwarfed immature *H. diminuta*; the large majority were completely negative, and the controls each had from 2–6 fully-developed adult *H. diminuta*. Age of infection with *H. nana* appeared to have no effect on the results. Simultaneous infection with cysticercoids of both species produces both dwarfed and normal *H. diminuta* and normal *H. nana*, the size of the *diminuta* apparently varying with the rate of development of the *nana*. Additional work to determine the relative effectiveness of *H. nana* eggs and cysticercoids in inducing this cross-immune reaction is in progress.

60. *Tetraphyllidean Cestodes from the Eastern Pacific*. C. G. ALEXANDER, University of California, Los Angeles.

Approximately 54 species of Tetraphyllidea have been recovered from sharks and rays collected during the past two years on the coasts of California and Baja California and in the Gulf of California. Up to the present, 21 species of selachians have been examined. The tapeworms have been provisionally referred to the following genera: *Anthobothrium*—11 species; *Acanthobothrium*—16 species; *Echeneibothrium* (or *Rhinebothrium*)—8 species; *Phyllobothrium*—10 species; *Scyphophyllidium*—1 species. In addition, one species of the diphyllidean, *Echinobothrium*, and 3 species of the lecanicephalan, *Disculiceps*, have been recovered. The majority of these forms represent undescribed species of known genera. Two additional species apparently represent undescribed genera of the family Onchobothriidae.

Forms which must be included in the genera *Phyllobothrium* and *Echeneibothrium*, according to available descriptions of the two groups, in many cases show few of the characteristics

of the more well known species of these groups. It is hoped that a thorough study of these forms may more clearly delineate speciation and affinities of the numerous forms presently referred to these two genera.

61. *The Biology of Oochoristica and the Status of Linstowiine Cestodes.* R. E. MILLEMANN AND C. P. READ, University of California at Los Angeles.

The life cycles of *Oochoristica* sp. from kangaroo rats and *O. scelopori* Voge from lizards have been studied. The cysticeroids of these forms develop in larvae or adults of *Tribolium confusum*; the worm from kangaroo rats will also develop in adults of *Gnathocerus cornutus*. These findings are in agreement with Rendtorff's studies on the development of *O. symmetrica* Baylis (= *O. ratti* Yamaguti and Miyata) in tenebrionid and dermestid beetles. The development of cysticeroids of *Oochoristica* from kangaroo rats is very slow at room temperatures (ca. 22 degrees C.). At 28 degrees C. the rate of development is accelerated two to three fold.

It has been amply demonstrated that oribatid mites are intermediate hosts of anoplocephaline cestodes. The present findings strongly indicate that the linstowiine cestodes develop in coleopterous insects. These gross differences in biology, along with the recognized anatomical differences between the two groups, justify the recognition of the family Linstowiidae.

60. *Cysticercus fasciolaris in the Syrian Hamster.* WAYNE W. WANTLAND, Illinois Wesleyan University, Bloomington, Illinois.

Repeated experimental attempts to infect the Syrian hamster, *Mesocricetus auratus*, with viable *Taenia taeniaformis* ova have failed. Ova pass through the digestive tract of this animal with the embryophore intact. Oncospheres with embryophores partly, as well as wholly digested away, were administered per os, injected into the blood stream, directly into the liver and into the body cavity. None of these methods resulted in infection of the hamster with *Cysticercus fasciolaris*. One accidental, and, as yet unexplained, infection with a single *Cysticercus fasciolaris* strobilocercus was noted at autopsy of a hamster from a separate series infected with *Trichinella spiralis*. The Syrian hamster resists infection with this parasite. The single case found in this study may have been due to the accidental ingestion of an extremely viable oncosphere.

(Accepted for later publication by the Journal of Parasitology.)

63. *Cysticercus fasciolaris in the Wild Rat and the Development of this Strobilocercus in the White Rat.* WAYNE W. WANTLAND, Illinois Wesleyan University, Bloomington, Illinois.

Examination of five hundred-thirty-two wild rats obtained from the city dump area of Bloomington, Illinois showed the livers of four hundred twenty-eight (80.45 per cent) animals infected with *Cysticercus fasciolaris* strobilocerci. Cysts found in individual rats varied from one to thirteen in number and ranged in size from two to nine millimeters in diameter. No evidence of liver sarcoma was found. A high incidence of infection in cats of this area with the adult stage of this parasite was demonstrated. *Taenia taeniaformis* developed in experimental cats after feeding of cysts from captured rats. White rats fed large numbers of ova developed extremely heavy infections which were tolerated very well. Such extreme invasion of the liver with low mortality of the host demonstrates the high factor of safety of liver tissue. As infections progressed tremendous eosinophilic necrosis and marked fibrosis was observed with areas of abnormal cells proliferating from, and in both close and remote association with, the capsules surrounding strobilocerci. Studies of cellular and tissue changes in infected livers of one hundred white rats over a period of fourteen months resulted in the detection of eight mixed-cell sarcomas and four spindle cell sarcomas. Evidence from this study indicates that 1.) the onset of the sarcomatous condition of the infected liver is preceded by pronounced eosinophilic necrosis and fibrosis and 2.) some factor or agent in addition to the parasite (*Cysticercus fasciolaris*) is required for the incitation of these types of liver sarcoma.

64. *The Occurrence of a Nymphal Linguatulid (Arthropoda: Pentastomida) in the Lungs of the Domestic Cat.* L. M. YUTUC, College of Veterinary Medicine, University of the Philippines.

The nymphal form of *Armillifer moniliformis* var. *heymonsi* had been recorded in man, monkey, tiger, leopard, Indian civet, domestic dog, Indian otter and Philippine civet cat. The adult had been reported from the reticulated python (*Python reticulatus*) in the Philippines and Indian python (*Python molurus*) in India. The present single specimen has been recovered at autopsy from the lungs of a cat, encysted under the visceral pleura. At first it was thought to be a gnathostome, a common helminth of cat but on closer microscopic examination

its true nature was revealed. Morphologically the juvenile arthropod is indistinguishable from the one described in the civet cat the Philippines. This is the third one to be recorded in the Philippine Islands.

65. *Statistical Analysis Applied to Numbers of Worms Recovered by Post Mortem Examination.* JOHN R. EGERTON, Kansas State College.

During the course of recent investigations it was discovered that the numbers of worms recovered from experimentally infected chickens follow the distribution described by the negative binomial $(q-p)^{-k}$ and that the analysis of variance as generally applied to the observed numbers is not a valid analytical procedure since in order to make tests of significance the data must follow a normal distribution. Consequently, a transformation which may be applied to observed values in order to "normalize" the data was sought.

Haldane (*Ann. Eugen.*, 11: 179. 1941.) presents a method of calculating the parameter k (where: $k' = -k$) by the maximum likelihood equation

$$N[1n(k' + m) - 1n k'] = \frac{n_1 + n_2 + \dots + n_R}{k'} + \frac{n_2 + n_3 + \dots + n_R}{k' + 1} + \dots + \frac{n_R}{k' + R - 1} \quad (1)$$

where r equals the observed number of the r^{th} class, n_r equals the relative frequency of r , R is the maximum value of r ,

$$N = \sum_{r=0}^R n_r, \quad m = \frac{1}{N} \sum_{r=0}^R r n_r,$$

and $E(m) = kp$ where $p = q - 1$.

If the reciprocal of the calculated k value is calculated as

$$\frac{1}{k'} = k_0 \quad (2)$$

then the value of k_0 may be used in the transformation

$$x' = k_0^{-\frac{1}{2}} \sinh^{-1}(k_0 x)^{\frac{1}{2}} \quad (3)$$

given by Beall (*Biometrika*, 32: 243. 1942.) where x equals the observed value and x' is the transformed value. In his paper, Beall has a table giving values of x' for k_0 ranging from 0.00 to 1.00 and x ranging from zero to 300. He also presents an equation which may be used for obtaining values of x' which fall outside the range of the table and advances an argument for the validity of analyses performed on data transformed as stated.

66. *How Parasites Tolerate their Hosts.* E. R. BECKER, Iowa State College. Presidential Address.

67. *Observations on the Lethal Action of Polyborate on Swine Kidney-Worm (Stephanurus dentatus) Larvae in Soil.* JOSEPH E. ALICATA, University of Hawaii. (See abstract #89.)

68. *Coccidiosis of Mammary Tissue in the Water Shrew, Sorex palustris navigator.* R. M. CABLE, Purdue University; and C. H. CONAWAY, University of Wisconsin.

Coccidiosis of the mammary gland in the water shrew was encountered by one of us (C. H. C.) in an investigation of the reproductive cycle in animals collected in Montana. In each female, one mammary gland was removed and fixed for histological study later. Three of 9 post-partum females proved to be infected and the parasites were found only in lactating individuals whose mammary tissue showed signs of regression. All stages typical of coccidian infections in epithelial tissues were observed. Although the mammary acini of one animal were denuded and filled with oocysts, fresh material was no longer available when the infection was detected and the sporulated oocysts essential to identification were not obtainable. Whether the coccidian is primarily a parasite of mammary tissue or an intestinal form that can develop facultatively in the mammary gland is unknown. The fact that only lactating animals were observed to be infected suggests that transmission may be associated with nursing by the young shrew. Although oocysts could be obtained in this process, it is difficult to conceive how the parasites might again reach mammary tissue.

✓ 69. *Contributions to the Life Cycles of Gorgoderid Trematodes.* WILLIAM H. COIL, The Ohio State University.

Two natatory, noncystocercous cercariae were found to develop in the viscera of unionid clams. These cercariae differ from all other gorgoderid cercariae by the possession of the following combination of characters: 1) a stylet; 2) a natatory, filamentous tail which has no anterior chamber into which the trematode could encyst or withdraw; 3) cystogenous glands both surrounding the bladder and scattered throughout the body; 4) development in the viscera of unionid clams; 5) a three-host life cycle.

These cercariae maintain themselves in the water by extending and contracting their tails in a manner similar to that of a bucephalid larva. They are swept into the second intermediate host, a clam, by means of the incurrent siphon and presumably penetrate the gut and migrate to the region of the gonads where they encyst. Encystment was traced in carefully-controlled experiments. The stylet, whose shape and size is characteristic for the cercarial species, was observed in the cystic fluid, and this corroborates the experimental evidence. Very few metacercariae were found in clams harboring sporocysts, indicating that some immunity probably is present. These distomes encyst slowly; a thin, delicate cyst is formed within a week. Later, further cyst material is added which cannot be differentiated by ordinary stains.

70. *Cercaria tiogae, a New Rhopalocercous Form from the Clam, Alasmidonta varicosa (Lamarck).* JACOB H. FISCHTHAL, Harper College, State University of New York, Endicott, New York.

Cercaria tiogae, a new species of rhopalocercaria, was found emerging from the freshwater unionid clam, *Alasmidonta varicosa*, collected from Catatonk creek, Tioga County, New York. Differentiation from known species is based primarily on the pattern of distribution of sensory papillae over the body surfaces. *C. tiogae* has 2 or 3 papillae on the posterior margin of the acetabulum, 8 (rarely 9) on the dorsal surface posterior to the acetabulum, and usually 11 (occasionally 12, 10 or 13) on the ventral surface posterior to the acetabulum. Furthermore, *C. tiogae* possesses 9 pairs of penetration glands while within the daughter sporocysts (latter located within the gonads of the clam). Upon emergence into the water, the cercarial tail transforms into an ovoid, non-adhesive, balloon-like structure, lacking a posterior filament. Cercarial encystment occurs within the chamber of this transformed tail. The metacercaria differs from the cercaria within the daughter sporocyst only in having one pair of penetration glands less, and also in lacking cystogenous glands.

71. *Life History of a Strigeid Trematode, Hysteromorpha triloba (Rudolphi, 1819) Lutz, 1931.* ERNEST J. HUGHINS, South Dakota State College. (See abstract #17.)

72. *The Infection of Duck and Goose Embryos by Plasmodium berghei, a Mammalian Malarial Parasite.* R. BARCLAY MCGHEE, The Rockefeller Institute for Medical Research. (See abstract #10.)

73. *New Host and Distribution Records for the Trematode Genus Braunina Heider, 1900.* ALLEN MCINTOSH, Zoological Division, U. S. Bureau of Animal Industry, Beltsville, Maryland.

Last January Mr. F. G. Wood, Jr., Curator, Marine Studios, Marineland, Florida, requested identification of a globular parasite (about 6 mm. in diameter with a small, short, cylindrical hindbody) found attached to the walls of the first and second stomachs of the bottle-nosed porpoise. The parasite was *Braunina cordiformis* Wolf, 1903, the only species of the family Brauniniidae Bosma, 1931.

The previous records are: (1) Heider (1900) who proposed the generic name *Braunina*, without a trivial name for the species, from specimens from two dolphins, one of which was dissected at the Zoological Station at Trieste in 1877, the other captured at Lesina and deposited in the Innsbruck Museum, and also on some material said to have been from *Squalus* captured near Rio de Janeiro; (2) Wolf (1903) who had fresh material from a dolphin collected at Trieste on Easter, 1902. Wolf after comparing his new material, on which he based his morphological studies, with specimens from "*Squalus*," collected on the voyage of the "*Novara*," decided that not only was the latter host incorrectly labeled—macroscopic and microscopic examination of attached pieces of host tissue being comparable to similar tissue from the dolphin—but that the parasite was undistinguishable from the one from the dolphin, *Delphinus delphis* Linn.

The specimens from Florida are from a mother and daughter (immature) of *Tursiops truncatus* (Montague, 1821). Additional specimens, from *Stenella graffmani* Lönnberg, 1934, collected by Dr. A. O. Foster on March 23, 1937, from Panama Bay, R. P., have been examined.

74. *Exogenous Proliferation in a Larval Taeniid (Cestoda: Cyclophyllidae) from the Body Cavity of Peruvian Rodents.* MARIETTA VOGEL, Department of Infectious Diseases, School of Medicine, University of California at Los Angeles.

Clusters of cysticerci lying free in the body cavity of several *Ctenomys peruanus*, were identified as belonging to the same species of the cestode family Taeniidae. The number of cysticerci in each cluster varied from 9–20, and their length ranged from 9–15 mm. Each cysticercus is connected by a slender, short stalk to a central vesicle from which it originated. Scolices are invaginated and bear about 20 hooks of similar shape, ranging from 190–214 μ in length. Sucker diameter range is 190–258 μ .

That this method of asexual, larval multiplication does not represent an abnormality but is part of the normal development of this species, is borne out by the relatively large number of this type of larva obtained from several host individuals.

75. *A Method for Revealing Cuticular Structures of Nematodes.* JULIUS FELDMESSER AND A. L. TAYLOR, Division of Nematology, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture.

Certain structures of nematodes (phasmids, cervical papillae, amphids, excretory pores, etc.) are sometimes difficult to locate but are important for purposes of identification. A technique involving relief staining has been devised to serve as an aid in microscopic studies of nematodes. Specimens intended for study are transferred to water or formalin solutions of china blue stain for periods varying from two hours to over-night. The particles of stain aggregate in depressions, pits and cavities, revealing these structures in relief. Lantern slides showing some of these features have been prepared.

76. *Infectivity and Host Response in Root-knot Nematode Infections.* V. H. DROPKIN, The Naval Medical Research Institute.

Infectivity and gall size were measured in an effort to find additional characters in genetic studies on *Meloidogyne incognita* var. *acrita*. Techniques were developed for quantitative studies on these characters. Results of measurements on cucumber and tomato seedlings are presented.

77. *Some Observations on the Monoxenic Cultivation of Certain Rhabditid Nematodes.* ELLSWORTH C. DOUGHERTY, University of California at Berkeley.

In the past five years hitherto unrecorded observations have been made on monoxenic and axenic cultures of certain species of the genus *Rhabditis* (*sensu lato*). I have maintained 3 species, generally at 18° C., on nutrient agar slants for varying periods in monoxenic culture with *Bacterium coli*—*R. briggsae* (over 5 years), *R. pellio* (3½ years), and *R. elegans* and the *micro* mutant of *R. briggsae* (1½ years). By contrast, *R. dolichura* larvae do not mature on this medium. All the others save *R. elegans* maintain themselves readily, even if transferred as infrequently as every several months; but the latter usually persist only if transferred at least monthly, surviving larvae from somewhat older cultures almost always maturing into sterile adults. *R. elegans* does much better on beef liver infusion agar. By contrast *R. briggsae* establishes itself only with difficulty on such rich media; yet, at the opposite extreme, it matures but does not reproduce with *B. coli* growing on mineral-glucose agar. The interaction of *B. coli* and substrate is thus important in providing a suitable medium for monoxenic cultures of these forms. Probably *B. coli* alone is not sufficient nutritionally for *R. briggsae* and *R. elegans*, and additional factors must be supplied by the medium. On certain media *B. coli* probably elaborates inhibitory substances or grows in a form difficult of ingestion. Any one or more of these factors could explain the differences between growth of *R. elegans* and *R. briggsae* on the various media tested. Probably *R. elegans* is more exacting nutritionally than *R. briggsae*.

78. *Some Observations on the Axenic Cultivation and Attempted Cultivation of Certain Rhabditid Nematodes.* ELLSWORTH C. DOUGHERTY, University of California at Berkeley.

Among rhabditids only *R. briggsae* has been recorded as grown with complete success under axenic conditions; partial success only has been reported with *R. pellio* on liver preparations. As yet unreported have been certain studies with *R. pellio* and *R. dolichura*. (As regards *R. elegans* the only series so far attempted proved contaminated.) Certain media on which *R. briggsae* grows well have been newly tested for *R. pellio*. The latter grows to large size on chick embryo pieces and in a medium consisting of liver protein fraction C plus the supernatant (ALH) from autoclaved liver homogenate, but, unlike *R. briggsae*, it does not reproduce in either. However, like *R. briggsae*, it reproduces abundantly on a medium containing chick

embryo juice plus certain known chemical substances. A single trial with *R. dolichura* has been made using imperfectly axenized larvae from a culture with mixed microbial flora; the medium used, unheated liver protein plus ALH, supported good growth and reproduction in a control series of tubes with *R. briggsae* while the *R. dolichura* larvae remained unchanged for a period of several days until contaminants developed. Observations in this and the preceding abstract thus suggest that considerable nutritional differences exist among the several species tested, *R. briggsae* seeming the least demanding. (These species are unequally related—in a forthcoming publication I am placing *R. briggsae* and *R. elegans* in one genus, *R. dolichura* in a second, and *R. pellio* in a third (*Rhabditis*, *sensu stricto*).)

79. *Axenizing and Monoxenizing Soil Nematodes*. ELLSWORTH C. DOUGHERTY, University of California at Berkeley.

The elimination of all associated bacteria from soil nematodes, growing in the presence of a mixed microbial flora, in order to establish the worms in axenic or monoxenic culture is often quite difficult. Although I have had a measure of success with *R. pellio* and *R. briggsae* using streptomycin and penicillin treatment followed by 1:1000 merthiolate, even this technique has proved unsatisfactory with these and other species, in some cases clearly because of the particular bacterial species involved. A modification that has given more reliable results involves following the antibiotic-merthiolate treatment by migration of larvae for several hours, or overnight, on a nutrient agar plate on which a thin layer of *Bacterium coli* has freshly grown. Apparently bacteria that may have survived the previous treatment are left behind as the larvae crawl through, and feed on, the colon bacilli. Success in monoxenizing *Rhabditis elegans* was only achieved by the foregoing method after several failures with simpler techniques. However, I have so far failed to axenize *R. dolichura*. It is possible that bacteria are protected more by association with certain nematode species than by that with others.

80. *Infectivity for Japanese Beetle Grubs Retained by Neoaplectana glaseri after Seven Years Axenic Culture*. NORMAN R. STOLL, The Rockefeller Institute for Medical Research.

Tests for infectivity of the normal host were made in 1951 with a strain of the nematode maintained in axenic stock cultures, serially, for over seven years. Originally isolated and rendered bacteria-free in Dr. R. W. Glaser's laboratory in 1944, it was received by the experimenter in Feb. 1948 after an estimated 132 generations in 44 monthly transfers on rabbit kidney or chick embryo tissue (Glaser, 1943). It has been carried similarly since, principally on rabbit kidney, with intermediate refrigerator storage of the dauer larvae.

In a second 44-month period to Oct. 1951 the strain continued vigorous and flourishing after 21 transfers and an estimated 63 additional generations. At that time Japanese beetle grubs became infected, after exposure to 3rd stage larvae in soil, and after being fed by mouth with droplets of water containing the larvae. Some grub cadavers showed large numbers of the nematodes, the results of apparently characteristic fatal infections.

This retained infectivity after an estimated 195 generations axenically *in vitro* is ascribed to the adequacy for this species of the kidney tissue type of cultures, and their inoculation with numerous larvae (100 or more), permitting maintenance of an essentially wild, heterozygous population.

Certain aspects of the results have bearing on the status of *Neoaplectana glaseri* as a parasite.

81. *Prenatal Infection of a Calf with the Nematode, Neoascaris vitulorum*. HARRY HERLICH AND DALE A. PORTER, U. S. Regional Animal Disease Research Laboratory, Auburn, Alabama.

Attempts were made to infect calves with *N. vitulorum*. Infectivity of eggs was determined by tests with white mice, eggs proving to be as infective 14 days after incubation at 28° C. as at any subsequent time over a period of 9 months. Seven calves from 2 to 180 days old were fed single or multiple doses of 1,000 to 108,000 infective eggs. Fecal and post-mortem examinations revealed that none became infected. In attempts to produce prenatal infection, seven pregnant cows were fed eggs totaling from 815 to 124,000 during the latter third of their gestation period. The number of egg doses ranged from 1 to 20, spaced over 1 to 62 days. The calves dropped by these cows were not infected with *N. vitulorum*. Two additional cows, Nos. 214 and 288, were fed 38,800 and 41,800 eggs, respectively, in 17 doses, from impregnation until parturition. The calf of Cow 214 was killed when 5½ weeks old and no worms were found, prior fecal examinations of this calf showed no ascarid eggs. Cow 388, dropped twin calves, both of which were negative for 22 days. Calf 1 continued to be negative for 3 months. Twenty-three days after birth, Calf 2 passed *Neocascaris* eggs which were fertile. Four days

later, a gravid female of *N. vitulorum* was passed in the feces. Subsequent fecal examinations were negative for eggs, and no more worms were passed.

82. *Transmission of Trichinae to Swine Through Feces.* LLOYD A. SPINDLER, U. S. Bureau of Animal Industry.

Swine normally acquire infections of trichinae by consuming garbage containing uncooked, infested pork or the carcasses of trichinous animals. In the past, certain investigators have believed that swine may acquire trichinae by consuming feces of animals that have eaten trichinous meat. To test this possibility, pigs, dogs, cats, and albino rats, designated as donors, were fed trichinous pork. Trichina-free swine were then fed feces eliminated by these animals 1 to 4 days after infection, the approximate period during which the intestinal trichinae were developing to maturity. Other swine were fed feces eliminated by the donors during the period 4 to 28 days after infection, which encompassed the period they harbored adult trichinae. Control swine were fed feces eliminated by other pigs, dogs, cats, and rats fed nontrichinous pork. The test and control pigs were slaughtered 4 weeks after the last feeding of feces and the diaphragms examined for trichinae by the digestion technique. The donors were examined also.

At necropsy, 27 of 29 pigs fed feces eliminated by trichinous donor animals 1 to 4 days after infection, harbored from 1 to 12,373 trichinae per diaphragm; 26 of 32 pigs that consumed feces eliminated by the donors 4 to 28 days after infection, harbored 1 to 3,750 trichinae per diaphragm. The donors all harbored massive infestations of trichinae.

Twenty control pigs fed feces of animals that consumed nontrichinous pork were trichina-free at necropsy, as were the donors.

83. *Occurrence of Amidostomum in Canada Geese.* C. M. HERMAN, Patuxent Research Refuge, U. S. Fish and Wildlife Service and E. E. WEHR, Zoological Division, U. S. Bureau of Animal Industry.

The gizzard nematode, *Amidostomum anseris*, has been reported frequently as the cause of losses among domestic geese in Europe. Cram's (1925) report of an outbreak in domestic geese in New York State was the first evidence of this worm in North America and since then there have been several reports of its occurrence in many parts of Canada and the United States.

There have been a few cases of *A. anseris* reported from Canada goose (*Branta canadensis*) and one report of *A. spatulatum* from this host. The authors have reported *A. anseris* from goslings from Michigan and Utah.

Beginning in 1948 we have had opportunity to examine the gizzards of a large series of Canada geese from several localities in the United States, mostly wintering birds from the coast of North Carolina. *A. anseris* was the only species identified from examination of a representative sample from our collection of gizzard worms. A high incidence of infection with *Amidostomum* was observed in geese from many localities in the United States, including all the major flyways. In most cases only a small number of worms was recovered from each individual but birds from a refuge in North Carolina, which was studied intensively because of periodic winter losses, yielded significantly higher numbers of worms per bird. The mean number of worms per individual from most areas was under 15 while from this specific refuge the mean was in the seventies each of the past three years, with one bird having over 1500 specimens in its gizzard.

84. *The Incidence of Some Common Canine Intestinal Parasites.* FRANK A. EHRENFORD, Pitman-Moore Co., New Augusta, Ind.

Dogs obtained from the states of Indiana, Illinois, Kentucky, Michigan, Ohio and Tennessee in various numbers were examined for intestinal parasites. Animals having appropriate parasite burdens were tested with potential anthelmintics.

The fecal flotation technique used employed a sodium dichromate solution of specific gravity 1.36 by dissolving 790 grams in 1 liter of distilled water. After addition of the sodium dichromate solution to the sedimented sample the commixed specimen was not centrifuged.

Examination of 2 to 3 fecal flotations per dog for a total of 377 dogs showed that 98.7 per cent had intestinal parasites. Specific helminthic and coccidial incidence, given in per cent, were as follows: *Toxocara* 24.9, *Toxascaris* 5.3, *Trichuris* 70.2, *Uncinaria* 16.9, *Ancylostoma* 50.9, *Strongyloides* 1.5, *Isospora rivolta* 71.8, *Isospora bigemina* 0.7, *Taenia*, 45.3, *Dipylidium* 16.1, Negative 1.3.

Examination at autopsy of 186 of these dogs showed that *Physaloptera* occurred in 30.1 per cent. One dog harbored 372 *Physaloptera*, two-thirds of which were attached in the duodenum within 2 inches of the pyloric valve, while the remainder were scattered in the stomach.

Observations at autopsy indicate that of the two ancylostomids, *Uncinaria* and *Ancylostoma*, *Uncinaria* produces much less damage than does *Ancylostoma*.

The distribution of helminths in the gut follows a general pattern, however, a great deal of variation occurs, e.g., one dog had approximately 10 *Taenia* attached in the colon.

85. *An Experiment on the Pathogenic Interaction of Haemonchus contortus and Nematodirus spathiger in Lambs.* K. C. KATES AND J. H. TURNER, U. S. Bureau of Animal Industry.

Various workers have demonstrated that a severe anemia in ruminants results from heavy *H. contortus* infections, often causing death; diarrhea and marked loss in condition are not usually associated with uncomplicated clinical haemonchosis. The writers (1949, 1951, 1953) demonstrated experimentally that heavy, pure infections of *N. spathiger* can produce an enteritis in lambs, which is usually accompanied by diarrhea and retardation in growth, but not by anemia or fatalities. Furthermore, at the last meeting Turner *et al.* (Abstr. 11) reported data suggesting that clinical haemonchosis acquired by lambs on pasture may influence the course of naturally acquired *N. spathiger* infections.

The combined effects of these two species were studied experimentally by administering sublethal doses of *H. contortus* and *N. spathiger* larvae to lambs, and comparing the effects produced by these mixed infections with those produced by similar pure infections of each species in comparable lambs. In the lambs with mixed infections, the characteristic clinical effects of each species were enhanced by the presence of the other; for example, diarrhea was more severe than in lambs fed only *N. spathiger* larvae, and the anemia more acute than in lambs fed only *H. contortus* larvae. The lambs with mixed infections either died or were near death during the course of the experiment, whereas those with pure infections of one species or the other were not as severely affected.

86. *The Effect of Cortisone on the Development of the Immune Response in the White Rat to Nippostrongylus muris.* P. P. WEINSTEIN, National Institutes of Health, Bethesda, Maryland.

White rats were given 5 immunizing doses of *Nippostrongylus muris* larvae over a period of weeks by injection into the skin of the back; shaved belly skin was then exposed to these larvae which were allowed to penetrate. Biopsies of the belly skins were then taken at the end of 48 hours and the animals necropsied 11 days after this last exposure. These rats had been divided into several groups as follows: Group 1. Rats injected with 2 mg. cortisone daily, beginning injections prior to the first larval exposure and continuing cortisone until necropsy. Group 2. Immunized to larvae; no cortisone. Group 3. Given immunizing larval doses, but started on cortisone 5 days prior to belly skin exposure and given cortisone until necropsy. Suitable controls were also studied. Rats immunized to larvae but not given cortisone showed intense inflammatory response in skin sections with many larvae trapped in nodules. Groups 1 and 3 given cortisone showed virtually complete suppression of cellular response in skin, and no larvae were found. The mean number of worms recovered from group 1 was significantly higher than that from group 2 in each of two experiments; however, cortisone did not prevent the stunting of adult worms nor did it suppress the formation of antibody as measured *in vitro* by the development of excretory pore precipitate in serum. The greater number of worms developing in cortisone-treated animals appears to be related to the suppression of the cellular response, thus not hindering their migration to the gut.

87. *The Effect of Pteroylglutamic Acid, Vitamin B₁₂ and Related Compounds on Ascaridia galli Infections in Chicks.* GERALD BRODY, Michigan State College.

A simultaneous deficiency of vitamin B₁₂ and pteroylglutamic acid (PGA) using a highly purified synthetic diet resulted in increased worm numbers and worm length in single comb white leghorn chicks infected with 500 embryonated eggs of *Ascaridia galli*. A single deficiency of PGA only resulted in increased worm numbers whereas a vitamin B₁₂ deficiency alone allowed only an increase in worm length. A deficiency of vitamin B₁₂ in chicks fed a 70% soybean oil meal diet infected with 500 embryonated *A. galli* eggs produced the same results as had been observed using the purified synthetic diet.

In addition to 100 mgm. per cent vitamin C to a synthetic diet deficient in both PGA and vitamin B₁₂ significantly decreased both worm numbers and worm lengths. In the presence of PGA, vitamin C still maintained this effect, but in the presence of vitamin B₁₂, regardless of the presence or absence of PGA, the addition of vitamin C had no effect. Thus it would appear that vitamin C can in part replace vitamin B₁₂ activity on the parasite. In a similar experiment using leucovorin in place of vitamin C it was found that leucovorin could replace PGA with respect to reduction of worm numbers with vitamin B₁₂ having no effect on worm numbers.

The addition of vitamin B₁₂ to a basal diet containing leucovorin in place of PGA did not significantly reduce the length of the worms.

The belief that leucovorin is the active form of PGA and that vitamin B₁₂ participates in the formation of leucovorin from PGA in chicks seems to explain the observed effects of PGA and vitamin B₁₂ on the infections of *A. galli*. If one considers PGA and vitamin B₁₂ to be biologically equivalent to leucovorin, it follows that a deficiency of leucovorin would result in increased worm numbers and increased worm length, an observation demonstrated in these experiments.

88. *Certain Net Effects of the Free-choice Administration of Phenothiazine to Sheep*. A. O. FOSTER, U. S. Bureau of Animal Industry, Beltsville, Maryland.

Eleven years ago (April 22, 1942), following two years of small-scale experimentation, the experimental sheep flock at the Zoological Division station, Agricultural Research Center, Beltsville, was started on a program of parasite control by the free-choice administration of phenothiazine in salt, 1 to 9. The only break in the regimen was an experimental interruption for 165 days at the start of the sixth year.

There have been no discernible ill-effects attributable to the drug, as determined from data on breeding, fertility, lambing, lamb growth and viability, wool production, and general condition of the flock. On the other hand, parasites have been well controlled, although not eradicated, as evidenced by periodic egg-count surveys of the flock, by *post mortem* recovery of worms from occasional animals that died or were sacrificed, and by an absence of mortality or morbidity attributable to parasites. Only when the regimen was interrupted, after five years of continuous reliance upon it, were losses sustained from parasitism. These losses were checked when therapeutic doses were given and the regimen reinstituted. Despite residual infestations of ten potentially injurious nematode species, these appear to have provoked no immunity that was adequate to protect when the regimen was discontinued. On the other hand, from the standpoint of practical parasite control, there appears to have been no evolution of phenothiazine-fast strains, although egg counts have increased during recent years. No other flock has been kept so long under exposure to the drug and none other has presented so crucial a test of the long-term influence of the measure.

89. *Observations on the Lethal Action of Polyborate on Swine Kidney-Worm (Stephanurus dentatus) Larvae in Soil*. JOSEPH E. ALICATA, University of Hawaii.

"Polybor-3" (Pacific Coast Borax Company, Los Angeles) a combination of sodium pentaborate tetrahydrate and sodium tetraborate pentahydrate, when applied dry or sprayed on soil at the rate of 5 pounds to 100 square feet, has been found lethal to kidney-worm larvae mixed with soil up to a depth of 1 inch. The percentage of viable larvae recovered from treated soil in contrast with corresponding untreated controls, was 9.7 to 15.1 percent at 10 days after treatment and 0.1 to 0.2 percent at 20 days after treatment. Rabbits fed infected treated soil or larvae recovered from such soil 10 days after treatment showed only a few liver lesions at necropsy one month later. No lesions were found in rabbits or a pig fed treated infected soil 20 days after treatment. All control animals showed extensive liver lesions at necropsy. Kidney-worm eggs were mixed with treated soil at intervals up to 28 days after treatment. During this time, the soil was exposed to light rainfalls. The eggs hatched normally, but the larvae died in a few days without reaching the infective stage. In one experiment, however, where the eggs were mixed with soil 35 days after treatment, and the soil had been subjected to heavy rainfalls, the larvae developed normally to the infective stage. No ill effects were noted in a young pig kept for one month in a small enclosed area which was treated twice with "Polybor-3."

90. *The Effects of Some Phenothiazine Derivatives and Analogs on Horse Strongyle Developmental Stages in Feces*. NORMAN D. LEVINE AND VIRGINIA IVENS, Univ. of Illinois, Urbana.

When mixed with horse feces, phenothiazine killed or prevented the development of the eggs or larvae of small strongyles (Nematoda: Strongylidae), in a concentration of 0.025 M. Of 11 phenothiazine derivatives studied under the same conditions, only 1,3,7,9-phenothiazine tetrasulfonic acid, 5,5-dioxide, tetrapotassium salt was active; it killed or prevented the development of larvae in a concentration of 0.1 M. Derivatives inactive at this concentration (the highest tested) were 10-ethylphenothiazine, 10-acetylphenothiazine, 10-isobutyrylphenothiazine, 10-caprylylphenothiazine, 10-hendecanolyphenothiazine, 10-lauroylphenothiazine, γ -oxo-10-phenothiazine butyric acid, ethyl ester, γ -oxo-10-phenothiazine butyric acid, butyl ester, 10-ethylphenothiazine-5-oxide and 10-ethylphenothiazine-5,5-dioxide. Of 9 phenothiazine analogs tested, pyocyanin dihydrochloride was active at a concentration of 0.005 M., phenazine at 0.01 M., phenoxathiin and chlorophenoxathiin at 0.025 M., and phenoxazine and xanthidrol at 0.05 M.

Xanthone, anthrone and phenoxathiin-10,10-dioxide were inactive at 0.1 M., the highest concentration tested.

91. *Illustration of Critical Phases in the Development of Litomosoides carinii, Filarial Parasite of the Cotton Rat.* ETTA MAE MACDONALD AND J. ALLEN SCOTT, The University of Texas, Medical Branch, Galveston.

Certain phases of the development of *Litomosoides carinii*, the filarial parasite of the cotton rat, are more suitably studied with living material than with fixed preparations. Some of these phases are illustrated in a motion picture. These include the characteristic activity of worms of various ages and the movement and location of the larvae within the vector, the tropical rat mite. Technical methods of handling these worms are described and the results illustrated.

92. *Recent Developments in Methods for Maintaining and Transmitting Litomosoides carinii in the Laboratory.* J. ALLEN SCOTT AND ETTA MAE MACDONALD, The University of Texas, Medical Branch, Galveston.

Numerous requests have been received for information concerning details of methods for maintaining and transmitting *Litomosoides carinii* in the laboratory. This paper summarizes the methods now in use, pointing out details which must receive critical attention. Several new modifications of the methods and apparatus are presented.

93. *Infections Resulting from Three Necator americanus Larvae.* PAUL C. BEAVER, Tulane University.

Three filariform larvae of *Necator americanus* were placed on the forearm of 9 adult volunteers. Seven to eight weeks later, five of the nine began passing eggs. In one the eggs were infertile. Skin reaction was relatively mild but distinct in each instance. Eight of the group were without history of previous infection.

94. *Further Observations on the Incidence and the Inconstancy of Laboratory Findings in Enterobiasis.* WM. HUGH HEADLEE, Indiana University School of Medicine.

Using the cellulose adhesive tape method, 418 hospitalized orthopedic patients (children) were examined for the presence of pinworm infection. Examinations being made on six (consecutive) days, with negative results, before the patient was considered to be non-infected. On these individuals a total of 4260 examinations were made, an average of 10.2 (with a range from 1 to 61) per individual. Of the 418 persons, 162, or 38.8 percent, were infected. On the basis of the first examination only, 87, or 20.8 percent, were found to be positive, this representing only 53.7 percent of those in the group who were actually infected. An average of 17.8 examinations per individual were made on the 162 infected persons.

The data on 76 infected individuals who had been examined on six or more consecutive days before treatment was begun, show that on any one of the six days, only 35, or 46.1 percent, could be shown to be positive by the method used. The following percentages of infection were found on days one through six respectively: 37, 41, 46, 59, 51, and 46.

These data lend further support to our previous contention that, for this population group, on any one day, we will be able to detect only about fifty percent of the actual positives present. It is hypothesized that similar "positive-rate" data could be established for other population groups. This is held to be a very important consideration in the evaluation of any data in reference to the efficacy of therapeutic agents used against pinworm infection.

95. *Chromatography as an Aid to the Taxonomy and Phylogeny of Animal Parasites.* GEORGE W. RAWSON, Ciba Pharmaceutical Products Inc., Summit, N. J.

96. *The Influence of Antibiotics in Feed on Sarcoptic Mange in Pigs.* DOYS A. SHORB, U. S. Bureau of Animal Industry.

Six pigs, naturally infected with *Sarcoptes scabiei*, were divided into 2 groups of 3 each at 7 weeks of age. One group was fed a balanced ration, the other was fed the same diet fortified with 1.8 gram aureomycin and 1.5 mg. vitamin B₁₂ per ton. Scrapings from the ears of all pigs were examined for mites at weekly intervals. Examination of the scrapings showed no change in mite infestation of the pigs fed the regular diet, and in 3 months they gained 14, 23 and 29 pounds, respectively; those fed the fortified diet steadily lost mites and gained 40, 46 and 54 pounds, respectively, during that time.

In a second experiment, 8 uninfected pigs, 2 to 3 months old, were divided into 2 groups of 4 each. One group was kept in clean quarters and did not acquire mites; the other was housed with 3 mangy pigs and became infested in 3 weeks. Each group was then divided into 2 groups

of 2 pigs each. One group of infested pigs and one group of the clean ones were fed the regular diet; the other groups were fed the fortified diet. Observations were continued for 3 months. The uninfested pigs on the regular diet remained free of mites and gained 99 and 72 pounds, respectively; those on the fortified diet gained 96 and 97 pounds, respectively; all remained free of mites. The parasitized pigs on the fortified diet were less severely affected than those on the regular diet and lost mites more rapidly, as determined by scrapings, but both groups grew more slowly than the corresponding uninfested groups. The infested ones on the regular diet gained 81 and 49 pounds, respectively; those on the fortified diet gained 78 and 79 pounds, respectively. At termination of both experiments, no mites could be found in ears of pigs fed the fortified diet, while all those fed the regular diet harbored mites.

97. *Intermediate Hosts in Ascaris Infections.* J. F. A. SPRENT, University of Queensland Veterinary School, Brisbane.

The significance of rodents in the life history of ascaris parasites has been extended to *Ascaris lumbricoides* (human), *Toxocara canis*, *Toxascaris leonina* and *Ophidascaris filaria*. Sixty vigorously motile third stage larvae of *A. lumbricoides* collected from the intestine of a mouse infected with eggs 10 days previously, were swallowed in a gelatin capsule. One month later 1 gm. hexyl resorcinol was taken followed by a saline purgative; there was no evidence of infection. Dogs of various ages have been given minced tissues of mice previously infected with eggs of *T. canis*. In mice the larvae were observed to grow to a maximum length of about 0.45 mm. Ten days after infection of a 5 week old puppy 3 third stage larvae measuring 2.048–2.954 mm., were recovered from the intestine; a control animal harbored no larvae. The larvae of *T. leonina* grew to a maximum length of about 0.66 mm. in the tissues of mice. After feeding minced mouse tissues to cats both third and fourth stage larvae were recovered from the intestine, but relatively little growth was apparent at 20 days after infection at which time 4th stage larvae measured 0.721–0.736 mm. Larvae of *O. filaria* grew relatively rapidly in mouse tissues and reached a more advanced stage of differentiation. The larvae became encysted in the muscles of the forelegs but reached their maximum growth in the liver where they attained 13 mm. and caused severe and frequently fatal lesions by 3 weeks after infection. Infected mice were fed to an adult *Python variegatus*, but the animal has not yet been killed.

98. *Clinical Parasitism in Cattle in Georgia.* J. S. ANDREWS AND D. J. JONES, U. S. Bureau of Animal Industry; W. L. SIPPEL, Georgia Coastal Plain Experiment Station.

Between March 21, 1952 and April 9, 1953, 12 bovines from 6 months to 10 years old, from 9 Georgia farms, were found on post-mortem examination to be heavily parasitized with helminths, as a consequence of which the animals were extremely weak and emaciated. The majority were anemic and suffered from a severe watery diarrhea. Worm egg counts were of little value in making a diagnosis of parasitism as the cause of the syndrome. The total death losses were approximately 5 percent of the nearly eighteen hundred head of cattle involved.

The average number of parasites recovered per animal was nearly 141,000. The smallest number of worms recovered from a single animal was 4,990, of which 2,840 were *Cooperia punctata*, 1,000 *Trichostrongylus axei*, 600 *Ostertagia ostertagi*, 350 *Oesophagostomum radiatum* and 200 *Haemonchus contortus*, in addition to several hundred *Dictyocaulus viviparus*. The largest number recovered was 568,000, of which 532,500 were *O. ostertagi*, 29,250 *T. axei*, 6,250 *C. punctata*, in addition to hundreds of fourth-stage larvae of *O. radiatum* in the mucosa of the large intestine.

Among the factors involved in the causation of this parasitosis, were the following: (1) importation of cattle from other areas, the imported stock being more susceptible than the native stock to infection with parasitic nematodes; (2) presence near the drinking water of wet areas which became contaminated with nematode eggs and larvae; (3) poor pastures and inadequate supplemental feeding; (4) overstocking of pastures; (5) the failure of anthelmintics currently used to remove effectively species of trichostrongyles other than *Haemonchus* from the digestive tract of bovines.

99. *Preliminary Report on Endoparasites of Beef Cattle in Kansas.* L. W. DEWHIRST, M. F. HANSEN, AND J. E. ACKERT, Kansas State College.

Results of the first year study of a long term research project revealed that the genera *Cooperia*, *Ostertagia*, *Trichostrongylus*, *Oesophagostomum*, *Haemonchus*, *Bunostomum*, *Nematodirus*, *Trichuris*, and *Moniezia* are present in beef cattle in the Flint Hills area of Kansas.

Monthly egg per gram (EPG) counts on a herd of beef cows of varying ages maintained under range conditions indicated a low degree of infection which varied only slightly with the seasons.

Weekly EPG counts conducted randomly on calves from the above mentioned cows from

the time of birth to date showed a low initial count in the spring (calves averaged 2 months of age) and summer followed by a rise in the fall. During the winter the EPG counts decreased but rose the following spring.

Preliminary results on the calves indicate that there was a positive correlation in the winter between the average EPG counts and average maximum soil temperatures. It is not assumed that winter temperatures *per se* affected the parasites and thus the egg counts but rather it is suggested that these low temperatures exerted an indirect influence on the parasites through the quantity and quality of the feed consumed by the host.

100. *Further Studies of the Value of Phenothiazine, Free-choice, against Mixed Infections of Nematodirus and Haemonchus in Lambs.* J. H. TURNER AND M. L. COLGLAZIER, U. S. Bureau of Animal Industry, Beltsville, Maryland.

Pursuing an experiment involving the control of *Nematodirus* in lambs by the free-choice administration of phenothiazine reported by Foster *et al.* (J. Parasitol., 36 [6, sec. 2] abstr. 12), a further trial similarly devised was initiated to re-assess the effect of this drug on *Nematodirus* when found in association with *Haemonchus*. The results confirmed the experimental findings of the earlier report. Four lambs on contaminated pasture but with access to phenothiazine and salt (1:9) gained 8 lbs. more per animal during the 24-week experiment than did 4 lambs on a similarly contaminated pasture but with access to salt only. The lambs on the medicated mixture averaged 14 per cent higher hematocrits than did those on the non-medicated mixture, and had correspondingly higher hemoglobin levels. No deaths were experienced in the medicated group, whereas there was 1 fatality and another lamb "in extremis" in the untreated group at the termination of the experiment. The untreated lambs, unlike the treated, maintained a high egg count of both species of parasites for the duration of the trial. The difference in infestation was confirmed at autopsy by the ratio of worm burdens between the two groups; *Nematodirus* 4:1, *Haemonchus* 3:1. Carcass grades averaged "good" for the lambs on phenothiazine, and "medium" for those on plain salt. These observations indicate that a free-choice phenothiazine and salt regimen affords considerable protection to lambs exposed to heavy infestations of *Nematodirus* and *Haemonchus*.

101. *Photosensitization Keratitis in Young Goats Following Treatment with Phenothiazine.* F. D. ENZIE AND G. E. WHITMORE, U. S. Bureau of Animal Industry, Beltsville, Maryland. (Introduced by A. O. FOSTER.)

About 42 hours after treatment with phenothiazine, 15 of 28 Toggenburg kids exhibited symptoms of photosensitization characterized by bilateral conjunctivitis, lachrymation, photophobia, edema of the eyelids and periorbital skin, and opalescence or opacity of the cornea. The severity of the reaction varied among the several animals, but there was no apparent correlation between the age of the kids (3 to 10 weeks) and the degree of susceptibility. Mature goats that were dosed at the same time, however, were unaffected. No specific medication was administered, but the animals were kept out of the sunlight for a few days. There was gradual remission of the symptoms and lesions, and all animals were apparently normal one week after treatment. This reaction to phenothiazine has not heretofore been reported in goats, nor in other ruminants in this country.

102. *Toluene (methylbenzene) against Intestinal Nematodes in Dogs and Cats.* F. D. ENZIE AND M. L. COLGLAZIER, U. S. Bureau of Animal Industry, Beltsville, Maryland.

In critical trials from 1947 to 1953, therapeutic doses of toluene, or approximately 0.1 cc. per pound of body weight, removed 97 per cent of 272 ascarids from 15 dogs, 87 per cent of 6711 hookworms from 56 dogs, and 39 per cent of 2720 whipworms from 42 dogs. With the exception of occasional vomiting, the drug was well tolerated in all respects. Similar doses removed 99 per cent of 205 ascarids from 25 cats and all of 28 hookworms from 3 cats. Several of these animals vomited but there was no apparent nausea or inappetence. A consideration of all available information, including comparative data obtained in conjunction with the aforementioned trials, indicates that toluene compares favorably in efficacy and safety with n-butyl chloride as a general anthelmintic for small animals and appears to be superior to the latter in simplicity of dosage and range of action.

103. *Effects of Certain Anthelmintics on Lumen and Tissue Phase Larvae of Ascaridia galli (Schränk).* M. F. HANSEN, B. R. B. PERSAUD, AND J. E. ACKERT, Kansas State College.

Experimental infections with *A. galli* were established in 200 laboratory raised chicks by giving each of them 100 ± 10 embryonated eggs of this ascarid. Half of these infected chicks were used to test four commercial anthelmintics and the antibiotic, aureomycin. The other

half of the chicks were kept as untreated but infected controls. All treatments were begun eleven days subsequent to feeding the chicks the ascarid eggs because at this time most of the larvae are in the tissue phase. The dosages of the anthelmintics were those recommended by the manufacturer, whereas, the aureomycin was given either at the rate of 18 mgm./chick/day for eleven days or as a single dose of 32 mgm./chick. In some of the experiments there was a daily killing of chicks in the treated and untreated groups for a period of ten days subsequent to treatment, whereas, in other experiments treated and untreated groups were sacrificed as a group eleven days subsequent to treatment.

None of the anthelmintics or the antibiotic tested affected the numbers or the growth of the tissue phase larvae. A combination of nicotine and phenothiazine restricted the growth of the lumen larvae. This combination of drugs appeared to reduce the numbers of lumen larvae; however, this study has revealed the possibility that the anthelmintic action was directed only against the less vigorous individuals among the lumen larvae which would be gradually eliminated over a period of time. Only antimonyl potassium tartrate showed any promise of removing more lumen larvae than just those less vigorous individuals.

104. *A Histopathologic Study of the Anterior Small Intestine of Immunized and Non-immunized Mice Infected with Trichinella spiralis.* JOHN E. LARSH, JR., University of North Carolina and GEORGE J. RACE, Duke University School of Medicine.

The present study was suggested by a recent report that in old mice (5.4-6.1 months) the defensive mechanisms bringing about expulsion of adult worms at reinfection operated only in the proximal half of the small intestine (Larsh, *et al.*, J. Elisha Mitch. Sci. Soc. 68: 1-11).

Based on the previous findings, a similar experiment for the purpose of histopathologic study was performed. Certain of the immunized and control mice were necropsied at intervals from 12 hours to 14 days post-infection. The remainder of the mice were necropsied seven days post-infection. The adult worm counts showed that the immunized mice had developed the usual degree of acquired resistance; and the serologic findings were similar to those reported previously for our mice.

In the immunized mice, a mild inflammatory reaction involving all parts of the mucosa and submucosa was noted as early as 12 hours post-infection. Thereafter, the reaction became acute, reaching a zenith at four days. The predominant cell was the polymorphonuclear leukocyte. As the reaction subsided, the inflammatory infiltrate became mixed mononuclear in type (plasmacytes, lymphocytes, and macrophages).

In the non-immunized controls, the difference in the cellular response from that above was chiefly one of time and severity. The inflammatory reaction developed much more slowly, reaching a peak at about ten days.

In both groups of mice, the peak of the cellular reaction occurred shortly before a sudden loss of worms was noted in comparable groups in the earlier study. These results seem to indicate that cellular mechanisms are involved in this resistance. Evidence from other studies in our mice strongly suggest that such mechanisms come into play after the primary action(s) of antibodies.

105. *Passive Transfer of Resistance to Trichinella spiralis in Mice.* JAMES R. HENDRICKS, University of North Carolina.

The present study was undertaken to determine whether or not circulating antibodies, demonstrated previously, are protective in action.

Thirty mice, six weeks old, were used in the one experiment completed to date. Twelve of these were immunized by giving them three stimulating infections at three-week intervals. Four weeks later, six of these mice were exsanguinated, the serum recovered and pooled; the other six were saved to be used as immunized controls. Twelve mice, previously uninfected, were employed as experimental controls. They were each given intraperitoneally one injection of 0.5 ml. of serum collected from the six donors. The remaining six mice, previously uninfected, were not given a serum injection and thus served as non-immunized, untreated controls. The 24 mice were infected with 200 *T. spiralis* larvae and necropsied seven days later, at which time a count was made of the number of adults in the small intestine.

The average number of worms recovered from the six immunized controls (54.2) is significantly less than that from the six non-immunized, untreated controls (140.6). These differences are within the range noted in previous experiments. The average number of worms recovered from the 12 experimental mice (105.3) is significantly lower than that of the non-immunized, untreated controls (140.6) and significantly higher than that of the immunized controls (54.2).

It is clear from these results that resistance was demonstrated in the experimental mice.

In a study in progress, mice will be sacrificed at intervals and adult counts made in an attempt to prove conclusively the passive nature of this resistance.

106. *The Effect of Water Starvation on the Natural Resistance of Mice to Trichinella spiralis.* CHARLES H. CAMPBELL, University of North Carolina.

Three experiments have been performed to date to study the effect of water starvation on the natural resistance of mice to *T. spiralis*. Each experimental and control animal was infected orally with approximately 200 larvae suspended in 0.1 ml. of nutrient broth and 2.5% gelatin solution. Results were based on adult worm counts performed seven days post-infection. In Experiment 1, the experimental mice starved for 24 hours prior to infection harbored an average of 176 worms, while controls allowed water *ad libitum* harbored an average of 180 worms. From the experimental animals of Experiment 2 denied water for 4 hours prior to infection and 17 hours post-infection (total 21 hours), an average of 156 worms was recovered in contrast to an average recovery of 180 worms from the controls. In Experiment 3, the experimental mice from which water was withheld for 20 hours from the time of infection harbored an average of 137 adults, whereas an average of 184 adults was recovered from controls.

These observations indicate that the intestine of the mice denied water shortly before and/or for a period of 17-20 hours post-infection was less favorable for the establishment and/or maintenance of *T. spiralis* larvae than was the intestine of the mice allowed water *ad libitum*. This serves to emphasize the necessity of insuring an adequate water supply to obtain valid experimental results. Further work is planned to test the effect of other periods of water starvation on the natural resistance of mice to this parasite.

107. *The Use of the Collodion Particle Agglutination Test for Detecting Antibodies Formed in Response to Trichinella spiralis Infection.* CHARLES H. CAMPBELL, University of North Carolina.

The collodion particle agglutination test is based on the fact that collodion particles sensitized with soluble antigens are agglutinated in homologous antisera. Since this test has proved to be sensitive and easily read macroscopically in work with other infectious agents, the possibility of its use in *T. spiralis* studies is being explored.

In the preliminary work thus far performed, serum collected from a rabbit 30 days after an infection with 12,000 larvae was tested. Three experimental antigens were used. The first consisted of the acid soluble protein fraction of larvae, originally prepared for precipitin testing, and the second and third, respectively, were prepared by incubating larvae for 48 hours in double distilled water and physiological saline and afterwards discarding the larvae. Control tests were performed with normal rabbit serum and unsensitized collodion particles.

Using 1:4 dilutions of the antigens, a serum titer of 1:160 was detected with the protein fraction antigen, and a titer of 1:320 with the antigen from larvae incubated in distilled water. No agglutination resulted in tests using the saline solution in which larvae had been incubated.

These results indicate that antibodies occurring in rabbit serum following *T. spiralis* infection may be detected under these experimental conditions. The antigenicity of the distilled water antigen was probably due to the presence of worm products released upon the death and disintegration of the larvae, since the saline antigen, in which no break-up of the worms occurred, yielded negative results. Experiments are being performed to study further the usefulness of this test in experimental trichiniasis in mice.

108. *A Comparison in Mice of the Infectivity of Trichinella spiralis Larvae in Normal Saline Solution and in a Solution of Nutrient Broth and Gelatin.* JOHN E. LARSH, JR., JOHN W. MCKENZIE, BERNARD G. GREENBERG AND CHARLES H. CAMPBELL, University of North Carolina.

Following infection of a large number of mice with larvae in normal saline, many of the remaining larvae appeared to be non-viable. This would not affect the experimental results, since mice of the various groups are infected alternately. However, it seemed desirable to insure more uniform infections in all animals. Since the effect on the larvae might have resulted from mechanical damage caused by the pumping action of the syringe before removing each dose, a solution of greater density than saline was considered. It was decided to test a nutrient broth-gelatin solution modified from that recommended by Culbertson as an aid in obtaining uniform doses (J. Parasit. 28(3): 197-202).

In three experiments, the larvae were standardized at 200 per 0.1 ml. Every third dose of larvae withdrawn was inoculated into a mouse, suitably marked to indicate the dose received. The intervening doses were withdrawn and the number of larvae counted. In this way, a total

of 100 larval doses were removed from the tube. In the first and second experiments, respectively, the larvae were suspended in 0.85 per cent NaCl and nutrient broth plus 2.5 per cent gelatin. In the final experiment, two tubes of larvae were prepared, one with saline, the other with the nutrient broth-gelatin.

A much greater percentage of adults was recovered from the mice infected with larvae suspended in the nutrient broth-gelatin solution, and the adult worm counts showed less variation than those for the mice infected with larvae suspended in saline. Other observations of this study will be reported later after the data have been subjected to statistical analyses.

109. *The Effect of Storage at Low Temperatures on the Infectivity of Aspiculuris tetraptera* Eggs. K. F. CHAN, Columbia University School of Public Health.

A. tetraptera is a useful parasite for the routine screening of anti-oxyurid agents. The technique of infecting *Aspiculuris* however, is tedious and time consuming. By being able to store the eggs of this parasite, there would be available a continuous source of supply of materials for infecting animals, which would greatly facilitate a drug screening program.

The eggs used in these experiments were obtained from gravid *Aspiculuris* from the large intestine of mice. Three samples of eggs representing different stages of embryonic development were kept in distilled water at 1 to 4 deg. C. for varying lengths of time. (Group I: Eggs in segmented stages dissected from female worms; Group II: Eggs of Group I incubated at 27 deg. C. for 2 to 4 days until the development of motile embryos; Group III: Eggs incubated to full development, usually 5 to 7 days at 27 deg. C.) All groups of eggs were viable after 40 days of refrigeration and when returned to optimum conditions of moisture and temperature continued their embryonation. On feeding these embryonated eggs to mice, infection resulted from all groups of eggs. Preliminary results of Group III eggs indicated that the hatching rates did not appear to decrease after 40 days of storage at low temperatures. The eggs of this group were further incubated at 37 deg. C. for 5 to 7 hours before feeding to the animals, as this appears to be essential after prolonged exposure to the low temperatures. *Aspiculuris* eggs apparently withstand refrigeration well and it is likely that they survive a longer period than 6 weeks. Studies on their viability for more extended periods are in progress.

110. *In Vitro Culture of the Larvae of Ascaris lumbricoides suum*. T. D. PITTS AND G. H. BALL, University of California at Los Angeles.

Larvae of *Ascaris lumbricoides suum*, hatched by the centrifugation method described by Pitts (1948), have been cultured in a variety of media. The most successful results have been obtained using a specially designed culture chamber that enables one to observe the larvae without sampling the culture. The medium that has supported longest survival contains 3.0 grams yeast extract, 3.75 grams peptone, 3.75 grams glucose and 250 ml. human serum made up to 1 liter with Fenwick's solution (Fenwick, Dec. 1939). The medium is Seitz filtered to obtain sterility. Incubation is at 37°-38° C.

In sterile media of this composition up to 93.6% survival has been observed at 16 days, 21.2% at 32 days and 2.4% survival at 39 days. Culture populations have ranged from 57 to 1200 larvae.

Longest survival has been with media that have not been renewed during the culture period. Tests are in progress to determine the effect on survival of daily renewal of the medium from a reservoir flask connected to the culture chamber.

The average increase in length of larvae surviving 34 days was 20% as compared to an estimated average increase in the pig of 440% at 7 days when the larvae are in the trachea (Ransom and Foster, 1920). Loosened sheaths have been seen on a few of the cultured larvae, but no complete molting has been observed. Efforts are being made to bring about molting, the absence of which may be preventing growth of the larvae.

111. *Preliminary Survey of Helminthic Diseases in Thailand*. ELVIO H. SADUN, Division of International Health, Public Health Service.

A preliminary investigation was carried out in Thailand by simple fecal smear technique in order to determine the incidence of enteric helminthiases.

Over 7,000 persons, selected at random in 29 provinces, were examined. For the purpose of this study Thailand was divided into 4 sections: North, Northeast, Central plains and South.

In the North the incidence of infection varied from 29 to 69% in different provinces. *Ascaris lumbricoides* was the dominant pathogenic parasite with an overall average of 32%. *Trichuris trichiura*, hookworm, *Strongyloides stercoralis*, *Opisthorchis viverrini*, *Taenia* and *Enterobius vermicularis* were less common but present in every province. In the Northeast the incidence of infection varied from 63 to 81% in different provinces. *Opisthorchis viverrini*

was the dominant helminth with an overall average of 27%. Hookworm, *Ascaris lumbricoides* and *Trichuris trichiura* were also rather common (24, 16 and 9% respectively). In the Central plains the incidence of infection varied from 16 to 85% in different provinces. *Ascaris lumbricoides* was the dominant helminth with an overall average of 21%. Hookworm *Fasciolopsis* and *Trichuris* were less common (15, 6 and 4 respectively). In the South the incidence of infection was uniformly high in all provinces visited. Among school children it varied from 93 to 97% in different provinces. *Ascaris lumbricoides* was the dominant helminth with an overall average of 81%. *Trichuris trichiura* and hookworm were also uniformly common (58 and 51% respectively).

Egg counts and epidemiological studies according to age, sex and occupational habits were carried out and will be reported elsewhere.

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Minutes of the 42nd Meeting of the Council and 27th annual meeting of the American Society of Parasitologists were published in the February, 1953 issue of the *Journal of Parasitology*, vol. 39, pp. 113-117.

Summary of Treasurer's Report for fiscal year, Nov. 1, 1951-Nov. 1, 1952. Complete report on file in the Secretary's office.

1. Balance on hand, Nov. 1, 1951	\$ 4,677.85	
2. Collections from all sources, Nov. 1, 1951 to Nov. 1, 1952	12,167.26	
(includes dues from 759 members and 905 subscriptions as main items)		
		Total \$16,845.11
3. Total expenditures		12,030.58
(includes 3 nos. and supplement of Vol. 37 and 4 nos. and supplement of Vol. 38 as main items)		
4. Balance on hand, Nov. 1, 1953		\$ 4,814.53

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1926 Philadelphia	1934 Pittsburgh	1842 (New York, cancelled)	1949 New York
1927 Nashville	1935 St. Louis	1943 (No meeting)	1950 Cleveland
1928 New York	1936 Atlantic City	1944 Cleveland	1951 Chicago
1929 Des Moines	1937 Indianapolis	1945 St. Louis	1952 Ithaca
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1931 New Orleans	1939 Columbus	1947 Chicago	
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